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Fifty years on

NIT-PICKERS will point out that the Commonwealth Scientific and Industrial Research Organisation (CSIRO) is not fifty years old this week—the body founded in 1926 was the Council for Scientific and Industrial Research (CSIR), and the transition in name only came in 1949. This cannot be allowed to diminish the satisfaction that such a major scientific venture is still alive, well, and showing no obvious signs of senility. Elsewhere in this issue ten or so authors describe some of the aspects of CSIRO, past, present and future. (It is worth recording that we have noticed, not for the first time, that Australian scientists, when commissioned to contribute to *Nature* not only say yes without grumbling about how unreasonable the demands are and how immensely busy they find themselves all of a sudden, but also actually deliver the material requested . . . in good condition . . . ahead of schedule.)

One thing that strikes the visitor to Australia is the public enthusiasm which still prevails for science and technology. This enthusiasm is not, strangely, converted into an unusually large allocation of funds to R & D activities—little over 1% of the GNP goes that way. But of this figure, government's share is, in the words of the 1974 OECD Examiner's Report on Science and Technology in Australia, 'exceptionally high'. This report goes on to note that 'the total resources of CSIRO for research and development are not much less than those of all universities taken together, and greater than the R and D effort of secondary industry'. Under these circumstances it is inevitable that CSIRO should be the subject of endless questioning, criticism and attack. It is a large target and many of the things people say about it are probably true and mutually contradictory. But what the OECD Examiners, with no axe to grind, said, is worth looking at again, if only because, following their report, it seemed as if there was widespread agreement that their comments had been sensible, and deserved serious attention.

CSIRO, they said, may be big and sometimes seem monolithic, but the worst thing would be to split up its more than thirty divisions and distribute them amongst government departments. Within the past year we have seen encouraging signs that the scientific community can be mobilised against such piecemeal dismemberment.

The imbalance between CSIRO and universities, they said, seemed to be too large; but the balance should be redressed by strengthening university research rather than by weakening CSIRO. There is little expansion still left in the Australian university system, but probably what the examiners were thinking more of was the contracting out of more CSIRO-type pure research which has traditionally been done in house—maybe making room within the organisation for more applied research, where the future probably lies for CSIRO.

The examiners further noted that the structure of CSIRO did not necessarily make interdisciplinary research easy, and one may note parenthetically that this could be the reason why the organisation has been rather slow off the mark in employing social scientists. There are encouraging signs now, however, of new flexibility in the management and if this cuts down the previous high degree of autonomy of division chiefs, it is probable that most of them are happier in the new mode of having to talk to each other.

Finally, perhaps the weakest aspect of Australian science, and CSIRO is no exception, is the industrial dimension. It is by no means clear that the blame for this can be put on remote professors or on too-single-minded division chiefs. Australian industry (or secondary industry as it is often called to distinguish it from agriculture and mining) is very frequently controlled from outside the country. The bright idea is sent over from some industrial laboratory in Europe or North America, and all the fellows down-under are expected to do is implement it. This has made for a difficult climate for industrial research and the problem is not easily soluble, even in the short term.

The founders of CSIR much admired the British Department of Scientific and Industrial Research but added a research spirit more akin to that of a university. Australia has profited greatly by this in the past fifty years, and the rest of the world has things to learn from CSIRO. Not least is the way in which career structure is founded on ability, not on length of service. In these days of obsession with job security the UK might turn to Australia, fifty years on, to learn one or two things about how to employ scientists. It would probably turn out to be a sobering exercise. □



Genetic manipulation to be patented?

A patent application governing commercial uses of recombinant DNA techniques has been filed in the United States. Colin Norman reports.

WHILE the scientific community has been loudly debating the potential hazards and benefits associated with a new technique for manipulating genes in living organisms, Stanford University and the University of California have been quietly trying to patent the technique in the United States. Rumours about the patent application, which apparently has been pending for at least 18 months, surfaced at a scientific symposium held at the Massachusetts Institute of Technology earlier this month; they were confirmed by university officials last week.

Though the people concerned with the patent application are reluctant to discuss it in detail while the matter is pending, the patent is understood to be worded broadly enough to cover commercial uses of any method of transplanting genes from one organism into another. The patent, if awarded, would not affect research uses of the technique, and it would apply only in the United States.

The basis for the application is research published in 1973 and 1974 by groups led by Stanley Cohen at Stanford and Herbert Boyer at the University of California, San Francisco. Their efforts led to the first demonstration that genes can be snipped from the DNA of virtually any organism and spliced into a bacterial plasmid (a ring of DNA which reproduces inside a bacterium independent of the bacterium's chromosomes). The key part of their research showed that the modified plasmid could be reinserted into a bacterium so that the transplanted, 'foreign' genes are copied each time the bacterium reproduces. It is understood that the patent application covers the process for constructing hybrid DNA molecules capable of self-reproduction, which means that, if awarded, it would also apply to transplanting genes into viruses and bacteriophages.

There has been speculation that the technique might, eventually, lead to

such commercial uses as the insertion into bacteria of genes capable of producing pharmaceutical products, such as insulin, so that specially engineered bacteria would be capable of secreting expensive drugs. Another speculative commercial application would be to graft genes capable of fixing nitrogen into crop plants such as wheat, to produce a new variety capable of synthesising its own nitrogen fertilisers from the atmosphere. Such uses of the technique would be covered by the patent application.

Discussion of the patent application arose at a symposium on genetic manipulation held by Miles Laboratories at MIT on June 8-10. Noting that there have been persistent rumours that somebody is trying to patent the technique, a speaker asked whether any participants could shed some light on the matter. Cohen, who was present at the meeting, confirmed that Stanford and the University of California are looking into the possibility of taking out a patent, and he emphasised that neither he nor any of the other researchers involved would benefit financially if it were awarded.

A patent application can be filed in the United States up to a year after the information on which it is based has been made public. The first paper by Cohen and Boyer's groups was published in November, 1973, which indicates that the application should have been filed before November 1974. Asked last week whether that assumption is correct, a Stanford patent officer replied that "it would be reasonable to assume that we were prudent in filing our application". In some countries, such as Great Britain, a patent must be applied for before any public disclosure is made. Britain's National Research and Development Corporation initially looked into the possibility of seeking a patent on genetic manipulation techniques developed by Kenneth and Noreen Murray at Edinburgh University, but dropped the idea because of prior disclosure.

When the matter was raised at the Miles symposium, two concerns were discussed. First, some scientists were worried that the patent, if awarded, might interfere with research. And second, it was suggested that it may

force some commercial concerns to seek a less safe, but patent-free, genetic manipulation technique. Neither concern seems to be valid, however, and in fact the patent, if awarded, could have some safety benefits.

As for the research implications, the patent would apply only to commercial use of the technique—it would not cover either academic or industrial research uses. Moreover, asked whether it may hold up beneficial applications of the technique, a Stanford official argued that royalties derived from the patent would be "reasonable" and would not limit its use.

As far as safety implications are concerned, the application seems to cover the key steps in the genetic manipulation process and thus, unless it is drastically narrowed by the US Patent Office, it would be difficult to see how a different patent-free process could be developed. If the patent is awarded, Cohen suggested at the Miles symposium that Stanford and the University of California could insist that commercial users of the process be required to sign an undertaking to abide by safety guidelines laid down by the National Institutes of Health (NIH).

NIH is due to issue guidelines on June 23 governing genetic manipulation research which it supports. At a meeting earlier this month, NIH Director Donald Frederickson briefed a number of industry officials on the guidelines, and though they met with general support, some drug company representatives expressed reservations about one or two provisions. The NIH guidelines, moreover, would not be binding on industry, since they would apply only to NIH-sponsored research. The patent may, therefore, provide a means of extending the coverage of the guidelines.

Cohen and Boyer's work was supported by the National Institutes of Health, but the federal government is unlikely to have a stake in the patent. Stanford has a standing agreement with the Department of Health, Education and Welfare (HEW) which gives the university patent rights on inventions produced from research supported by HEW grants, unless the project has been exempted from the agreement. According to an HEW official, the genetic manipulation studies were not exempted. Stanford patent officers have, however, been discussing the application with federal officials, particularly as regards establishing licensing arrangements if the patent is awarded. □

Pakistan's ambitious nuclear power programme

from Azim Kidwai, Karachi

TWENTY-FOUR nuclear power plants operational in a developing country by the end of the century seems too ambitious a programme in the present global context, but that is the plan at the drawing-board stage in Pakistan. Work on a second nuclear plant, at Chashma Barrage, on the river Indus, 150 miles south of Islamabad, starts in a few months' time. The project is estimated to cost Rupees 527 crores (527 million dollars). The 137-MW nuclear power plant at Karachi has been in commercial operation for the past five years. The Chairman of the Pakistan Atomic Energy Commission (PAEC), Mr Munir Ahmad Khan unfolded the revised crash plan for 24 nuclear power plants recently, in the wake of a bilateral agreement with France that includes the offer by the French of a nuclear reprocessing plant (cost 150 million dollars) for Pakistan.

The rationale for such an unorthodox accelerated nuclear power programme can perhaps be found in the poor energy resources of the country, coupled with the present very low *per capita* electrical energy consumption.

The known fuel resources in Pakistan have been estimated to be only 13 tons coal equivalent (TCE) *per capita*. The figure presents a dismal picture when viewed against the world average *per capita* resources of 1,200 TCE. Similarly, the *per capita* electrical power consumption in Pakistan at present stands at 140 kWh as against 1,360 kWh world average, which has been extrapolated to be 6,500 kWh by the end of the century.

The requirements at the close of the century have been estimated at 27,000 MW (current production is only 2,570 MW, and the huge Tarbela Dam will soon add another 2,000 MW). Hydropower and gas—the only two energy sources of consequence in Pakistan — could only supply 13,500 MW. There seems not much of an option left, since planning on imports of huge quantities of oil would be hazardous. The main argument of PAEC, though, is that “the nuclear power plant's fuelling would be much cheaper than those of oil fired plants. The generation cost of a nuclear power plant would be 33% cheaper than that of a conventional power plant”. In the long run, seen against the recent oil crisis, the argument appears to have validity even taking the higher capital costs of nuclear power plants into consideration.

All this planning on nuclear power may undergo revision if oil is struck in large quantities in Pakistan. Tremendous efforts are on the way as the 1976-77 budget on oil exploration at Rupees 42 crores (42 million dollars) testifies. The allocation has been more than doubled as compared to the figures in the current year. But without oil, the main plank of energy strategy in Pakistan will have to be nuclear because of the good deposits of uranium discovered recently in Pakistan and soon to be exploited. Concerted efforts to locate more uranium deposits also continue. Some of the areas have also been found with sizeable extents of thorium-bearing mineralisation—this fits into Pakistani nuclear think-tank schemes which envisage branching off to thorium.

One nuclear plant each year, on average, is planned after the early 1980s. The scheme includes dual-purpose nuclear plants as well. For instance, feasibility studies on such a dual-purpose nuclear plant for Karachi were mounted some time back in which the city could add to its grid another 400 MWe by the early 1980s and could also augment its water supply by 100 million gallons of sweet water from the sea daily.

Such a vast programme of nuclear power development calls for considerable manpower with sophisticated technical know-how. The PAEC has been training a large number of scientists and engineers for the last decade to meet the present situation. There are already some 350 scientists and engineers trained abroad on the rolls of the PAEC and a large number have been or are being trained at the Reactor School at Nilore near Islamabad. Another big nuclear power training centre which will cost Rupees 2.5 crores is being planned. Based at Karachi, and designed to train about 200 persons including technicians and operators annually, the centre would fill the gap on the technical manpower requirements of the PAEC in the coming years.

USSR's polar research

from Vera Rich

ARCTIC and Antarctic research has long been a major field of Soviet research. Indeed, the establishment of the first *Severnyi Polyus* (North Pole) drifting ice-floe research station and the forcing of the North East Passage by an icebreaker in the 1930's had at that time much of the prestige value now associated with the space programme. This sense of the spectacular is still sometimes apparent in the announce-

ment of new Soviet Arctic projects.

The “Polar Experiment North-76” from April to July this year, for example, was announced as the “largest comprehensive expedition in the history of Arctic exploration”, involving ten research vessels (including the flagship *Professor Vize*), aircraft, and upper atmosphere probe rockets. According to Professor A. Treshnikov, Director of the Arctic and Antarctic Institute (Leningrad), the principal aim of the expedition is to calculate the thermal balance of the Arctic ice-cap simultaneously on land, in the ocean, in the atmosphere and on the drift-ice (including observations at the North Pole itself). The data gathered during the 100-day expedition will be used to formulate mathematical models of hydrological and meteorological processes for use in weather forecasting. The expedition is envisaged as the first in a series to take place at one- and two-yearly intervals as part of a programme sponsored by the World Meteorological Organization.

Results of the previous voyage of the *Professor Vize* are meanwhile being processed. That expedition, during the Antarctic summer of 1975-76, produced data indicating that the Antarctic circumpolar current is “equal in flow to six Gulf Streams”. The research vessel *Dmitrii Mendeleev* also visited the Antarctic during the same summer on its first biological expedition. According to the expedition leader, Dr L. A. Ponomareva, its programme included research into the fauna of the ocean bed, the sampling of water from the bottom layers of the Macquarie complex, study of the Macquarie and Hjort trenches, the collection of sedimentary and geological material and the construction of plankton profiles south from New Zealand and Tasmania to the Antarctic ice.

Also back from the Antarctic is the Twentieth Soviet Antarctic Expedition. The programme of this expedition appears to have followed the usual pattern of meteorological and glaciological observations. Innovations included the automated processing of data obtained from upper atmosphere rockets—the first time, it is claimed, that this has been done under Antarctic conditions—and a new way of drilling glaciers using a borehole filled with a “non-freezing liquid”. Drilling at the Vostok station was carried out to 450 m, and at the Novolazarevskii station (where no previous bores had been made) to 357 m—the total thickness of the icecap at this point. The TASS agency hailed this expedition as a notable achievement in international cooperation: it included medical workers and geodesists from East Germany; an American geologist

wintered at the Molodezhaya station; and a Soviet glaciologist wintered at the US McMurdo base. A regular exchange of information was maintained with the British, Australian, US and Japanese Antarctic stations.

In contrast to these large-scale undertakings, a modest expedition of six skiers set off at the end of April from Wrangel Island to make their

way over the ice to the drifting *Severnys Polyus-23* icefloe station. This expedition was somewhat misleadingly described by TASS as a "sports expedition", and although its close association with the newspaper of the young Communist movement at first suggests heroic adventure rather than serious research, this was not the case. One of the main aims of the expedition was

the study of problems of psychological compatibility in a small team working under extreme conditions. The rations of the party were based on those of cosmonauts, and the results of the project may be intended for use in the study of the potential personality conflicts and stresses which may arise aboard a space-station or in long space flights. □

correspondence

More on Paradisia

SIR,—It is good to see you airing the Paradisia/Dominatia relationship in your columns (May 20, page 178). As a labourer in a different corner of the vineyard from Professor Brock, I find myself with another perspective. My concern is the training given to young Paradisian Scientists (PhD students and post-doctoral fellows) when they visit Dominatia.

I think it is fair to assume that most Domination Scientists would like to help Paradisia develop and operate a self-supporting research effort—suited both to the intellectual and the material needs of the country—as soon as possible. I sometimes wonder, however whether they set about things the right way. There may be States where the system to be aimed for closely resembles one to be found in Western Europe or the USA, but such places must form a small minority.

The normal situation is one where at no time in his working life is today's young Paradisian academic likely to have the resources either of time (because of a heavy teaching commitment) or money to undertake research in a way comparable with his Domination counterparts. His high intelligence, diligence and determination cannot alter this fact, and it does no-one any good to attempt to ignore or disguise the situation. The ideal training for an individual of this kind is therefore different from that generally offered to natives of Dominatia. It needs, above all, to encourage independence and self-reliance (hard these days when research projects are often scarcely comprehended by a student before he starts to write his thesis). Experimental ingenuity with scarce resources and self-criticism allied with the optimism which attempts to cut a coat according to cloth (rather than weeping and complaining that good cloth is unavailable) are also essential.

In all of this Dominatia can help, but to be really useful involves more

than treating students from Paradisia in the same way as their British counterparts. Doing so sometimes produces a fine scientist by Domination standards, but also someone who is discontented and unwilling to adapt to a different environment in the Paradisia to which he returns. Ideally a Domination supervisor will take the trouble to learn about Paradisia—its scientific research facilities, the peculiarly Paradisian problems which science might help to solve, even the government policies relevant to science and higher education. He needs to view the collaboration as part of the continuing development of his Paradisian colleague's life, as well as a contribution to his own research group's output. He should even, perhaps, begin to question whether his own view of the purpose of research, and what is worthwhile research, is the only tenable one. It may be that work which is not publishable in his own favourite journal can nonetheless provide important answers in a different context. In our joint efforts to help Paradisia, it is not only the Paradisians who need to learn.

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Egypt

PhD in evolution

SIR,—Your Editorial "PhD in evolution" (May 27) seems in its penultimate paragraph to reveal a general misconception about the purpose and nature of PhD research. It is assumed that PhD research should provide training for scientists going into industry. This, however, is not the case. The chief motive of a student going to a university science department to do research should be a desire to take part in the advancement of science. If he really wants to be trained for industry, he should go into industry, where he will receive a better training for that particular job than universities can provide. It is true that

industry sometimes, not to say often, finds that PhD research has provided skills and attitudes which are useful to it, but this is not the primary purpose of PhD research.

Distorting university postgraduate research to provide a training explicitly and directly useful to industry will harm the progress of fundamental science and, conversely, a postgraduate research student entering a science department should expect to learn about fundamental research and the particular activities that entails. If he expects a course aimed at preparing him for industry he is embarking on university research with the wrong motive.

It may be said that, if PhD research is not providing a training for industry, there should not be many science research students. I think this view is correct; I believe university scientists should take on a research student only if he has a genuine desire to carry out original work in science and recognises that this may not help him to get a better job outside university.

These comments apply to PhD research in "pure" science departments; I believe the situation may be different in engineering and technological departments.

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The real AI

SIR,—Whilst bemoaning the antihumanistic potential of artificial intelligence (May 13, page 171), it is particularly inappropriate to make use of the long established and well recognised acronym for artificial insemination.

Yours faithfully,

DARRELL H. THORPE

London, UK

news and views

Discovery of superheavy elements

from Peter Hodgson

THE super-heavy elements 116, 124 and 126 have now been detected in the mineral monazite from Madagascar by a group of nuclear physicists from the University of Florida at Tallahassee, Oak Ridge National Laboratory and the University of California at Davis.

Over the past few decades a series of elements heavier than uranium have been discovered, mainly by successive addition of neutrons. After plutonium and neptunium came elements such as fermium, einsteinium, mendelevium, nobelium and lawrencium. All are highly radioactive, and the heavier they are the shorter their half lives.

It has been known for a long time that the stability of nuclei is connected with their shell structure, those with magic numbers of neutrons or protons (corresponding to complete shells) being particularly stable. Lead 208 is especially stable, consisting of 126 neutrons and 82 protons, both magic numbers. The results suggested that the nuclei with even higher magic numbers of neutrons and protons might also be more stable than surrounding nuclei. It was considered very doubtful whether they would actually be stable, but it was thought possible that they would have half-lives long enough for them to be detected and their properties established.

The ensuing search for the offshore islands of nuclear stability or near-stability in regions of the periodic table beyond the peninsula of the heavy elements captured the imagination of nuclear physicists. Many different methods have been discussed, and among the most promising of these is the hope that the super-heavy elements (as they came to be called) could be produced in collisions between heavy ions at energies high enough to overcome their mutual electrostatic repulsion. The difficulty is then to make them stick together, for there is so much energy in the system that they are very likely to fly apart again,

The possibility of such reactions was one of the strongest arguments put forward in support of the funding of heavy ion accelerators, and these are now operating or under construction in the USA, USSR, UK and other countries. At the same time there has been intense theoretical work devoted to the prediction of the properties of the super-heavy elements by extrapolation from the properties of the known nuclei. It is thus a great surprise that super-heavy nuclei have now been found to be stable and waiting to be found in a familiar mineral.

The presence of unusual elements in monazite was suggested by studies by Robert T. Gentry of the halos in mica caused by radioactive inclusions. Most of the halos could be identified as due to known radioactive nuclei, but a few could not. It proved possible to remove the inclusions from the mica and bombard them with 5.7 MeV protons from the Tallahassee Super FN Tandem. Examination of the resulting X-rays by T. A. Cahill, R. C. Flocchini, N. R. Fletcher, H. C. Kaufmann, L. R. Medsker and J. W. Nelson showed frequencies corresponding to the $L\beta_2$ transition in element 116 and the $L\alpha_1$ transitions in elements 124 and 126. These transitions were found only using the targets from inclusions showing the anomalous halos. These super-heavy elements are identified with 99% confidence, and in addition there is weak evidence for the elements 114, 125 and 127. The abundance of the super-heavy elements is about 10^{11} atoms per inclusion. Their chemistry is being studied by T. A. Carlson and B. Fricke. The alpha and beta activity of these elements is being studied by J. D. Fox and K. Kemper; the alpha particle energies are around 5 MeV.

These results have stimulated intense theoretical activity in a group consisting of J. Berelacqua, D. Stanley, P. J. Philpott, D. Robson, F. Petrovich and M. Golin. Using the single-particle shell model, it is possible to obtain the energies of the single-particle states as

eigen values of a one-body potential. The parameters of this potential can be fixed to give the energies of the known single-particle states in Pb, Sn and Ba. Using the known dependence of the one-body potential on A and on the nuclear asymmetry parameter $(N-Z)/A$ enables the energies of the single-particle states in heavier nuclei to be calculated. Calculations made some time ago indicated that $Z=114$ is likely to be the next nucleus of higher stability than its neighbours, so the experimental indications of $Z=116$ are somewhat puzzling. Calculations for $Z=126$ indicate that this also should be a stable nucleus, shown by the energy gaps between the filled and the next unfilled shells.

Calculations were also made for the nucleus $Z=164$ and this comes out to be particularly stable, with neutron and proton energy gaps of 2.9 and 2.4 MeV respectively. The energy of the alpha-particles from the decay of this nucleus was calculated to be 9.5 MeV, which corresponds very well with the unexplained 'giant halves' found in monazite. This nucleus has not yet been detected by the X-ray method, so this at present remains an unconfirmed possibility. If confirmed, it could account for the anomalous lead isotopic ratios found in monazite.

The alpha-particle half-lives were calculated for a range of super-heavy nuclei, and were found to be sufficiently large for them to be found naturally for elements 114, 124, 125, 126 and 127, but not for 116 and 128. So 116 is a puzzle in this respect as well. The element 164 was on the borderline of half-lives acceptable for stability.

There is certainly much more experimental and theoretical work to be done to confirm these results, to determine the structure of these new nuclei, and to account for their properties experimentally. This is a major breakthrough which will stimulate many new researchers in the months to come. □

Antiquity of tropical rain forests

from Peter D. Moore

It is often stated, and widely believed, that tropical rain forest is a vegetation type which has existed for a considerable length of geological time; some authors even suggest that no major evolutionary developments have taken place in such forests since Cretaceous times. In fact there is very little in the way of fossil evidence by which such claims can either be supported or refuted. Some new information, however, has been gleaned by Graham (*Evolution*, **29**, 723; 1976) as a result of a pollen analytical study of upper Miocene lignites from a lowland area of Coatzacoalcos, Veracruz, Mexico. A hundred and ten pollen and spore taxa have been identified from these lignites and on the basis of these identifications some comparisons can be made with modern vegetation. True tropical rain forest taxa are poorly represented as are the arid community representatives now found in the area, but instead components of oak or pine-oak forest are frequent with possibly *Picea-Abies* forest at higher altitude. Reconstruction of past communities on the basis of pollen assemblages is, however, fraught with problems, and as Graham gives little indication of the precise location of his sampling sites, the relative abundance of different taxa, or the methods used for determining palaeocommunities, it is difficult to appraise his conclusions critically.

Quaternary palynological work from other tropical areas such as the Galapagos (Colinvaux, *Nature*, **240**, 17; 1972), South America (van der Hammen, *J. Biogeog.*, **1**, 3; 1974), and northern Australia (Kershaw, *Nature*, **251**, 222; 1974) certainly indicate that considerable changes in vegetation have occurred within the Pliocene, though the reverse situation may obtain in certain areas, such as the Cuatro Ciénegas basin of Mexico (*Nature*, **247**, 129; 1974). It is not unreasonable to suppose, however, that changes were also proceeding within the Tertiary in some places. The vegetation of many tropical forests may have undergone adjustments in its composition, presumably in response to climatic changes, and the concept of community migration, which is so popular with temperate palaeoecologists may also have relevance in the tropical context. For example, the needle-leaved element in Graham's assemblage could represent an extension of the highland forests of Central America in response to lower temperatures.

Veblen (*Biol. Conserv.* **9**, 141; 1976) describes such forests with a high pro-

portion of coniferous trees from the highlands of Guatemala. *Abies* and *Pinus* are present in these forests, together with *Cupressus*, *Taxus*, *Taxodium* and *Podocarpus* and these may represent relict fragments of more extensive Tertiary coniferous forests. Indeed, the abundance of species in the genus *Pinus* (29 spp.) in the area has led Mirov (*The Genus Pinus*, Ronald Press, New York; 1967) to speculate that the highland areas of Guatemala and Mexico may have been a secondary centre of evolution for the genus.

The continued existence of these conifer-rich forests in Guatemala is of more than academic interest. Veblen points out that they represent an important genetic resource which is currently being eroded by exploitation for timber. The land is subsequently used for agriculture, so that little regeneration is possible (Gomez-Pompa *et al.*, *Science*, **177**, 762; 1972). Severe genetic depletion in the forest communities is a real threat in these conditions since genetic races of various species will inevitably become extinct even where the species itself survives. Such a process must be regarded as an economic catastrophe in the long term, for these forests could supply the species so badly needed for the expansion of forestry in the tropics. So far only one of the highland Guatemalan conifers, *Cupressus lusitanica*, is used for this purpose; it has been planted in Africa very successfully and seems to have a wide climatic tolerance. Its timber production in Africa is said to be eleven times greater than that of species native to the plantation areas.

Fortunately these conifer forests of the uplands of Guatemala contain a rare and attractive bird, the Quetzal (*Pharomachrus mocinno*) which is the surest ticket any habitat can have for a rescue conservation effort of international proportions. The efforts in particular of the International Union for Conservation of Nature and the World Wildlife Fund led to the establishment in 1973 of a reserve of more than 400 ha of high altitude forest on the slopes of Volcano Atitlan in south-western Guatemala (La Basille, *Biol. Conserv.* **5**, 60; 1973). According to Veblen, there is one other area where the Guatemalan forests seem to be fairly secure—the Department of Totonicapan, where the local Indian population has long exploited the forest in a balanced manner. To them the forest represents a source of income and it is therefore jealously guarded. It is a sad reflection on modern society that the future of such potentially useful plant communities, with some claim to antiquity, now hangs in the balance. □

Red Sea: poles apart

from Peter J. Smith

FOUR years ago, Girdler and Darracott (*Comments Earth Sci. Geophys.*, **2**, 131; 1972) drew attention very briefly to the possibility that the Red Sea may have evolved in at least two separate stages of seafloor spreading. In addition, they raised, but made no attempt to answer, the question of whether or not the two phases of plate movement involved could be described by the same pole of rotation. In later presenting the evidence for a two-stage development of the Red Sea, Girdler and Styles (*Nature*, **247**, 7; 1974) made no reference to the secondary question of rotation poles, contenting themselves with describing the nature and dating of the Red Sea's geological history. But the omission did not go unnoticed; and Richardson and Harrison (*Earth Planet. Sci. Lett.*, **30**, 135; 1976) have now rectified it with a conclusion not without geological implications.

According to Girdler and Styles, a depression may have existed in the Red Sea area as early as the Carboniferous and major rifting probably began during the lower to middle Eocene. The first phase of seafloor spreading then took place between 41 and 34 Myr ago, forming what is now the wider (southern) Red Sea (that is excluding the axial trough). The evidence for this spreading came chiefly from recognisable magnetic anomalies outside the axial trough and the timing was derived from best-matching the anomalies against different portions of the reversal time scale for different spread-



A hundred years ago

M. W. DEFONVIELLE has had a spectroscope constructed with a graduated screen permitting the quantity of light admitted to be diminished in a known ratio. The moving force being regulated at will, the radiometer can be put in a state of rotation under the rays of the most scorching sun and record taken of the motion very easily. With such an apparatus it was shown by comparison with a standard oil-lamp burning forty-two grammes an hour, that on June 9, at 4 o'clock precisely, the radiating force of the sun was equal to fourteen lamps at a distance of twenty-five centimetres from the axis of the radiometer. The apparatus is tried daily at La Villette gas-works, and results of the comparisons will be tabulated and discussed.

From *Nature*, **14**, 181, June 22, 1876.

ing rates. The best spreading rate was 1.4 cm yr^{-1} over the 7 Myr.

This first spreading episode was followed by 30 Myr of quiescence during which great thicknesses (up to 5 km) of sediments, mainly evaporites, were deposited. Then 4–5 Myr ago a second phase of spreading began, splitting the older deeper oceanic crust with its heavy sediment load and creating the axial trough with new oceanic crust (and magnetic anomalies) rising to within 2–3 km of sealevel. This spreading phase continues today, giving the Red Sea axis a high heat flow and making it seismically active.

The Girdler–Styles model vindicated Davies and Tramontini (*Phil. Trans. R. Soc.*, **A267**, 181; 1970) to the extent that their seismic evidence had suggested that most of the Red Sea was underlain by oceanic crust while many workers were still maintaining that the undoubtedly oceanic axial trough gave way to Precambrian shield rocks. At the same time, however, it gave additional force to the question raised earlier by Girdler and Darracott. In answering this question now, Richardson and Harrison accept the rotation pole at 36.5°N , 18.0°E for the total opening of the Red Sea, obtained by McKenzie *et al.* (*Nature*, **226**, 1; 1970) by fitting together the coastlines. They point out, however, that given the validity of the Girdler–Styles model this pole will be the resultant of the first and second episodes of spreading. The overall NE–SW motion for Red Sea opening then may or may not be applicable to either of the spreading stages taken separately.

The lines joining congruent points of the opposing Red Sea coastlines are, of course, parts of small circles about the McKenzie *et al.* pole of rotation. According to Richardson and Harrison, however, the lines joining congruent points on the opposing sides of the axial trough are parts of small circles about a different pole at 15.2°S , 32.8°E . The axial trough thus seems to have been formed not by a NE–SW separation as for the Red Sea as a whole but by a roughly E–W separation. Vectorial subtraction of the new axial trough pole from the overall pole of McKenzie *et al.* then gives a pole of rotation of 29.6°N , 20.6°E for the earlier phase of Red Sea spreading.

One consequence of this calculation is that the Dead Sea rift, widely accepted as a transform fault, lies closer to a small circle of the new earlier pole than it does to a small circle of the McKenzie *et al.* pole; in other words, it seems to be a better behaved transform fault than previously supposed. Richardson and Harrison believe this to be “more than a fortuitous occurrence” and argue that the earlier phase

of Red Sea spreading led to a chiefly left-lateral motion along the Dead Sea rift. But logic then dictates that the supposed change in direction of the second spreading phase should have imparted to the rift a large element of normal faulting. Whether such faulting can actually be observed is a matter of controversy, however. Most geologists familiar with the Red Sea area seem to dispute its existence, although a few claim to have seen its effects. It is a pity that Red Sea earthquake data are so poor that currently available fault plane solutions are incapable of resolving the question.

Genetic function of mitochondrial DNA

from D. Wilkie

An international conference on mitochondrial DNA was held at Riva dei Tessali under the auspices of the University of Bari from May 25 to 29, 1976. The organisers were C. Saccone and A. M. Kroon who will edit the proceedings to be published by North-Holland Publishing Co., Amsterdam. The publication date is July 30, 1976.

THIS was the latest in a series of Bari conferences on mitochondrial biogenesis which started in 1967. The predominant organism in this field continues to be the yeast *Saccharomyces cerevisiae* although contributions from those working with animal cells, trypanosomes, *Paramecium* and filamentous fungi are fast catching up and were well represented among the 35 papers presented. The main advantage in working with the yeast system is the availability of genetic analysis of mitochondrial mutants. After the discovery had been made that mitochondrial drug resistance markers recombine in zygotes, the Slonimski group at Gif assiduously applied themselves to the genetic analysis of mutants, the most recent being the class known as *mit*⁻ (“point” mutations in mtDNA) discovered by Tzagoloff and Needleman (New York). A further possible source of markers was suggested from evidence that mitochondrial factors can function in the regulation of nuclear genes (Evans and Wilkie, London). An up-to-date linkage map of the yeast genome was presented by Slonimski and Tzagoloff listing some 13 markers. If the *ts* mutant of Schweyen (Munich) and the RNA-coding segments are included (Rabinowitz, Chicago) reported 19 tRNA species identified so far, the

map becomes quite extensive and may account for as much as 50% of the total genes carried. The map obtained by genetic analysis corresponds well with results from deletion mapping in petite mutants using DNA–DNA hybridisation technique (Nagley and Linnane, Monash) and those obtained by following progressive loss of the genome at restrictive temperature in the *ts* mutant (Schweyen). In view of this progress, the mapping of genes in yeast mitochondria as an end in itself may no longer be justified and the emphasis should shift to the functional aspects of the markers. Since the pioneering studies of Roodyn and Work, it has been known that proteins are synthesized in mitochondria and yet a mitochondrial gene and its translation product remains to be identified in any detail. (Butow, Dallas) reported a correlation between mitochondrial mutants and variation in organelle proteins obtained by gel separation after labelling yeast cells and provided evidence of segregation and recombination of polypeptide species in crosses. (Grivell, Amsterdam) described attempts to identify mitochondrial gene products of yeast synthesised from mtDNA in a coupled transcription-translated system of *E. coli* but results were complicated by interaction of translation products with proteins of the system.

Extensive use was made of bacterial restriction endonucleases in the analysis of mtDNA. This technique of obtaining precise fragment patterns is a powerful tool in the construction of physical linkage maps of mtDNA of all sources, coupled as it usually is with DNA–RNA hybridisation. Dawid (Baltimore) used the technique together with the electron microscope, in a comparative study of mtDNA of *Drosophila*, *Xenopus* and man (HeLa) and concluded that there was considerable base-sequence diversion between the species, indicating evolutionary diversification. Saccone and Kroon (Bari and Groningen respectively) concentrated on the RNA from purified mitochondrial ribosomes of rat liver. Hybridisation of RNA species of the 5S ribosomes with mtDNA fragments allowed construction of a map of rRNA-coding segments which mapped some distance apart. Attardi (Caltech) working mainly with tRNAs of HeLa cell mitochondria, presented evidence that both light and heavy strands of mtDNA are transcribed into functional tRNAs. Symmetrical transcription of mtDNA necessitates a reappraisal upwards of our estimates of the coding capacity of mitochondrial genomes if this phenomenon is general. Preliminary results in other systems, for example of Rabinowitz with yeast,

were in support of this. The original application of restriction enzyme analysis of mtDNA, namely to detect differences in fragment patterns between strains and mutants of yeast, was extended by Barnardi (Paris) in an elegant demonstration of recombination of mitochondrial genomes in yeast crosses: physical evidence of recombinational events was clearly seen in vegetative segregants with new fragment patterns compared with those of the parental strains. On purely statistical grounds, Barnardi drew the interesting conclusion that cleavage sites of restriction endonucleases may be GC-rich segments of the genome which are not themselves coding regions. Four papers were concerned with the complexity of kinetoplast DNA of trypanosomes. Endonuclease treatment and electron microscope studies indicated that the strange network of KDNA may be made up of aggregates and concentrates of several thousand mini-circles which individually have a contour length of $0.3\mu\text{m}$. However, there may be more than one class of mini-circle as indicated by sequence heterogeneity, more than one sedimentation peak and little renaturation of mini-circle preparations.

The mtDNA of *Paramecium aurelia* is circular but the circles may vary in size (Cummings, Colorado). Borst (Amsterdam) could provide no evidence that the mtDNA of *Tetrahymena* is other than linear and showed that replication intermediates are also linear, so this organism remains the odd one out in this respect. Systems for transference of mitochondrial type from one cell to another in *Paramecium* (Tait, Edinburgh) and in mammalian tissue culture (Eisenstadt, New Haven) were described as well as a system for the expression of mouse mtDNA in prokaryotic and eukaryotic cells (Clayton, Stanford).

A million memories?

from Andrew Holmes-Siedle

SOLID-STATE physicists have been refining their views on how far the miniaturisation of semiconductor circuitry can go (Wallmark, *Institute Phys. Conf. Ser. No. 25*, 133; 1975 and Keyes, *Proc. Inst. Elect. Engng*, **63**, 740; 1975) and it is thus worth seeing how near the integrated-circuit industry is coming to the physical limits which have been defined. Such is the urgency of the competitive rush to cram circuits on to silicon chips in larger and larger numbers and to operate them at high rates that it would seem that, if the rate of size reduction maintained its pace, designers would soon face funda-

mental barriers such as the speed of light, the onset of avalanche breakdown in semi-conductors and the randomness of impurity distribution. The field of semiconductor memories is at the centre of this competition.

The electronics journals are pulsating with advice and controversy over which of the dozens of designs of silicon memory chip are the best to use. Chips measuring $0.3\times 3\times 3\text{ mm}$ and carrying 4,096 bits of information can now be made cheaply (say for £5) and there is speculation that next year the semiconductor industry may be able to mass produce a chip containing 16,384 bits with little increase in chip size. Both devices are in demand for installation into new minicomputers and, in addition, all the logical operations will soon be performed on a single "central processor" chip (the "microcomputer"). Also, advanced technology laboratories are already hard at work on the

example if a particular diffused junction region must have dimensions less than $2\times 2\mu\text{m}$). Several tricks in metal-oxide-semiconductor (MOS) device technology, however, have been devised which make it possible to pack in more functions per square micrometre without reducing the individual line widths. First, the use of multilayer conductor geometry enables two electrodes to overlap (Fig. 1); second, the surface of the silicon is now being etched into grooves (Fig. 2), which leads to the first example of a vertical MOS device, and third, the number of electrodes needed to perform a function is being reduced by the invention of new device principles. For example, using the memory principle shown in Fig. 3, the charge-coupled random access memory (CC-RAM) it seems possible for information to be stored in a cell of $100\mu\text{m}^2$ ($10\times 10\mu\text{m}$) or smaller. The cell structure is so simple that the individual line widths used need not be less than $3\mu\text{m}$. As 1 cm^2 is $10^8\mu\text{m}^2$, it is easy to get a million bits on to an area smaller than a thumbnail. In fact, allowing extra 'real estate' for leads, amplifiers and wiring contacts, the

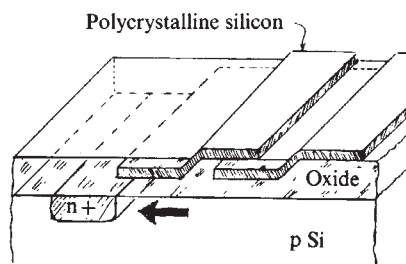


Fig. 1 Two level electrodes.

65,536-bit (2^{16} -bit) memory chip, which might be produced by 1979.

I wish to show that in this "memory chip" field, we may get all we need in the way of storing information in a small space without meeting limitations due to physical laws. I submit that we probably store about a million (2^{20}) bits on a single chip of silicon only just over half an inch on a side (say $15\times 15\text{ mm}$) without great efforts and that we will then probably stop trying to go further because this capacity is adequate for most purposes where miniature size and low weight are important, and the cost of even greater density may be prohibitive.

As I have discussed before (*Nature*, **257**, 739; 1975), several physical constraints come in when the width of an

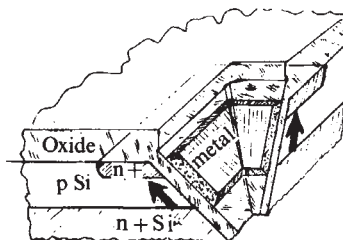


Fig. 2 Use of depth in Si.

individual line in the circuit pattern becomes less than about $2\mu\text{m}$ (for

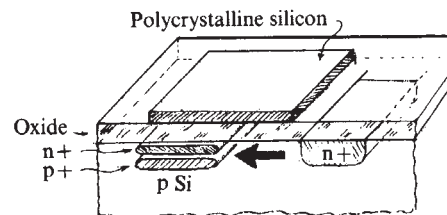


Fig. 3 Use of new electrodeless effects.

million-bit chip would probably be $15\times 15\text{ mm}$ or $0.6\times 0.6\text{ inch}$. If we consider about a thousand of these chips mounted in individual ceramic packages and then on circuit boards, and stacked in the corner of a room, we could easily imagine a capacity of a few billion bits of memory in a small cabinet. Would we want to go further in capacity in this form? Probably not; the *Encyclopaedia Britannica* only contains 40 million words and any larger storage requirement, say the keeping of archives or the storing of video information (a billion bits is only a few video frames), can quite often be handled by the use of disks or tapes.

This is only to say then that, in the semiconductor field, the basic device phenomena are available to take us to the small "mass memory" density needed in small, portable computers. There will probably be at least half a decade of technological effort before the million-bit density is achieved at reasonable cost. The technology which will challenge magnetic tapes will be of an entirely different character and will probably involve light holography. □

CSIRO: fifty years of research

CSIRO, the Commonwealth Scientific and Industrial Research Organisation of Australia, celebrates its fiftieth birthday this year. We present in this special supplement a cross-section of the activities of an organisation employing over 2,000 professional scientists. Sir Robert Price, Chairman since 1970, introduces the review.



Looking to the future

J. R. Price

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Although research in CSIRO will be determined by the significant advances in science as a whole, much emphasis will still be on the use of resources, the environment and consumer-oriented research. This is discussed against a background of increasing interaction between science and politics.

ALTHOUGH any attempt to assess the future role of an organisation such as CSIRO can only be highly speculative, it is valuable to try to do so even if nothing more is achieved than the identification of existing deficiencies. But whatever the outcome, it is necessary to devote a little time to considering some of the events that have contributed to the present situation in Australia with regard to science and technology and to the role of CSIRO.

A suitable starting point is the establishment, in 1926, of the Council for Scientific and Industrial Research (CSIR). Among its terms of reference was a major requirement that it initiate and carry out "scientific researches in connection with, or for the promotion of, primary or secondary industries". The Council was given considerable freedom to achieve its aims, and one of its first decisions—a crucial one—was that it should carry out its own research, with its own staff, in its own laboratories. To do this, it set up a number of research divisions and to this day the division remains the basic organisational unit of research in CSIRO.

Initially the emphasis of the Council's work was almost entirely on primary industry, and a succession of useful discoveries helped the new organisation gain acceptance by that industry and by the community in general. This encouraged the Government, in 1936, to provide resources to extend the activities of CSIR into areas of research for secondary industry. This proved to be a wise decision because of the part played by the newly established divisions in the wartime development of the Australian manufacturing industry.

Sir Frederick White deals in the next article with some aspects of those early years and has outlined the background to the transformation in 1949 of the Council (CSIR) into the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Referring to the early years of CSIRO, Sir Frederick writes of that period as one in which money was easy to obtain. It was a period, spanning the 1950s and most of the 1960s, of comparative prosperity and stability for Australia, a period which saw the setting up of the Murray Committee on Australian Universities

and the implementation of that Committee's recommendations. These met the pressing need for additional support for existing universities and led to the establishment of new universities and to a considerable expansion in university research. These important steps, which had the full support of the Executive of CSIRO—the Chairman, Sir Ian Clunies-Ross, was a member of the Murray Committee—led to a much improved balance in the national research effort between university and government laboratories and, incidentally, reduced a measure of undesirable friction which existed between some university scientific staff and CSIRO.

There was, and still is, however, a serious imbalance as far as industrial research is concerned, a situation which was bettered to a limited extent in that same period by the introduction of industrial research and development grants by the government, a step which was also actively supported by CSIRO. At present, then, the inadequate level of research activity in manufacturing industry is in marked contrast to the substantial proportion of the country's total research effort represented by government research agencies of which the largest is CSIRO.

By the late 1960s CSIRO had grown substantially, setting up new divisions and expanding and changing the direction of existing divisions, and in spite of very limited growth during the 1970s the total staff now exceeds 7,000, one-third of whom are professional scientists. As we moved into the 1970s, however, it became increasingly evident that CSIRO

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Nature is particularly grateful to Dr F. G. Lennox of the Australian Scientific Liaison Office, London for advice on the compilation of this supplement.

indeed, Australian science and technology as a whole—would have to adapt to a rapidly changing economic, social, and political climate. Similar pressures had already been encountered by governmental research organisations in other countries. In Britain, in Canada, and in the USA, for example, government-funded research agencies have, in the past decade, had to contend with a variety of problems, partly financial but significantly concerned with the relationships between science, perceived national needs, and often unperceived social deficiencies.

Nevertheless, there is no doubt that the most significant factor affecting Australian science and technology, and certainly CSIRO, in recent years, has been the growing involvement of science with politics. Politicians are becoming increasingly aware of science, and scientists can no longer count on the uncritical support of governments. A very important factor in bringing about this change was the establishment of a Ministry for Science—initially a Ministry for Education and Science.

Although this increasing interaction between CSIRO and government, and the growing need to justify policies and programmes, has not affected the integrity and quality of research in CSIRO, it can in some cases, and properly so, influence the choice of a particular research programme or the relative levels of support in certain areas.

A criticism of CSIRO which one encounters from time to time is that it is too large. Those who offer this criticism rarely state what they mean by “too large”—whether for effective management or because the Organisation receives what is thought to be too large a slice of the financial cake provided for science and technology by government. Yet in the size of CSIRO lies one of its great strengths. The sense in which I use the word ‘size’ relates not just to numbers of people employed or to budgets but to the wide diversity of scientific and technological activities embraced by CSIRO’s programmes.

This aggregation of scientific skills and facilities is not only exceedingly valuable now but will become even more so in the future, for two reasons. First, because of the growing importance of collaborative multi-disciplinary research which is most easily achieved within one organisation. Collaboration with other bodies, both nationally and internationally, is of course still important and will be even more so in the future. Such collaboration, however, tends to operate on a more formal basis and cannot always be entered into quite so readily or operated so efficiently. Second, because of the broad base of activities there is almost always a nucleus in one or more divisions which can be developed and built on to initiate research on new

problems arising from changing community needs or changing economic conditions. These two reasons constitute a very strong case for the retention of a broadly based governmental research organisation such as CSIRO, although there will always be a need for other more specialised bodies.

Against that background let me try to assess the role that CSIRO may have as government and community demands on scientists change, as change they must.

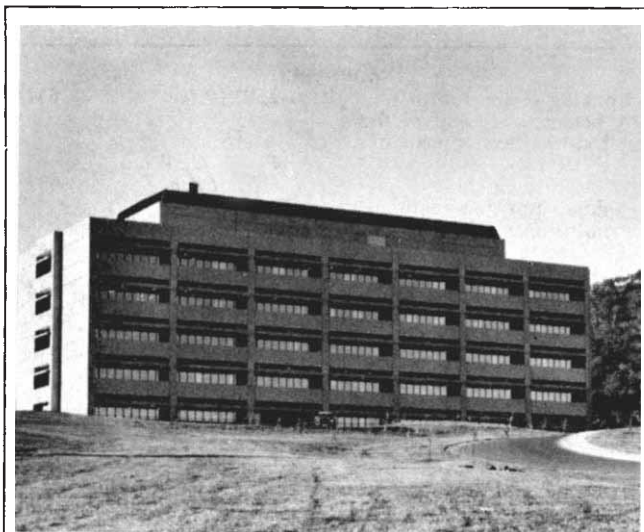
I repeat that the broad base on which CSIRO operates will make it easier to cope with changing needs with minimal delay or upheaval. These changing needs will not create problems different in kind from those which CSIRO has already faced. It is unlikely, however, that CSIRO will, in the foreseeable future, experience growth rates comparable with those of the 1950s and 1960s. Consequently it will need to develop the ability to redeploy resources in response to changing priorities to a degree that has not been necessary in the past.

But it is not sufficient to consider CSIRO in isolation. It is only one component in the total structure of scientific research in the Australian community and it must maintain and improve relationships with other components. A criticism of CSIRO which I think has some validity is that during the period of expansion to which I have referred the Executive was unduly inward looking, perhaps understandably in the circumstances. The changes which have taken place in the 1970s have forced on us acceptance of the need for more effective external relations with various official bodies and community groups—in particular, with industry, with Departments of State, with universities and with other bodies either involved in research or having research requirements, including the Australian Science and Technology Council. At present, CSIRO supports research in universities on only a very small scale and only infrequently gives research contracts to industry. There are good reasons why the letting of research contracts by CSIRO to universities, industry and other institutions, should be expanded considerably, and I envisage that this will occur in the future.

Although the role of CSIRO will continue to be one which rests essentially on the application of the natural sciences, there is no doubt that the problems of the future require an increasing input from social scientists. The Organisation has already recognised this by recruiting small numbers of social scientists into Divisions such as Land Use Research and Building Research. I envisage a considerable increase in recruitment of social scientists, their wider distribution throughout CSIRO, and a wider spread of socially oriented programmes across divisions.

I do not propose to make predictions in this article about those fields of research on which CSIRO is likely to concentrate in the future, although it is clear that there will be emphasis for a long time to come on such obvious areas as resources, renewable and non-renewable, and on environmental and consumer-oriented research. The directions of CSIRO’s future research programmes will be determined by the significant areas of advancement of science as a whole, by the requirements of the country as seen both by the scientists and the politicians as expressions of community needs, and by the practicalities of the human resources and material facilities available.

In the final analysis, the Executive is responsible to Parliament for ensuring that the Organisation’s resources are used efficiently and, in terms of the needs of the Australian community, effectively. The Executive’s ability to achieve this in the future will hinge on CSIRO remaining an elitist organisation recruiting the best research scientists it can on a worldwide basis, on providing the best possible facilities consistent with the resources available, and on maintaining within the Organisation an environment that is conducive to creative and productive research.



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A personal account of the historical development of CSIRO

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The origins of CSIRO can be traced back to the foundation in 1916 of the DSIR, under the pressure of scientists, for a greater use of science in industry. After some initial uncertainty as to how to run such an organisation, CSIR was set up in 1926. Early research was devoted to agriculture, but in the late 1930s CSIR re-directed its thrust towards industrial research. Under a McCarthy-like scare in 1949, the CSIR's name was changed to the CSIRO and since then it has continued to produce important work on a wide variety of topics.

AUSTRALIANS were first stimulated to form a national research institution by the news of the proposal in 1915 in Britain, during the First World War, for greater use of science for national efficiency and progress. A deputation representing learned societies, led by Sir William Crook's, President of the Royal Society put this proposal to the British Government; later in that year A. J. Pease, President of the Board of Education, announced to the Commons a scheme for an advisory committee for scientific research. Arthur Henderson, who became the President of the Board of Education when the government changed, presented as a White Paper to both Houses of Parliament *A Scheme for the Organisation and Development of Scientific and Industrial Research*. By Order-in-Council of July 28, 1915, a Committee of the Privy Council for Scientific and Industrial Research and an Advisory Council were formally established. It was from these beginnings that there emerged the Department of Scientific and Industrial Research (DSIR) whose excellent activities many scientists in Britain and overseas remember. It set a pattern of Government sponsorship of research in science; DSIR generously assisted younger and similar institutions in what we used to call the British Empire.

Because of the initiative of prominent academic leaders, one of whom was David Rivett's predecessor as Professor of Chemistry in Melbourne, David Orme Masson FRS, an Advisory Council for Science and Industry was set up in 1916 by the Australian Government of Mr W. M. (Billy) Hughes. Although it had wide terms of reference it did not succeed in establishing continuing national research. Its one success, however, the support of research on local hardwood timbers led, eventually, to the development of the pulp and paper industry.

By 1920 it was apparent that the Advisory Council was not an effective arrangement. The responsible members of the Council, led by Orme Masson, eventually, though with difficulty, persuaded the Prime Minister to pass a Bill creating the Institute of Science and Industry (1920). The powers and functions specified in the Act are similar to those written into the Science and Industry Research Act which created the Council for Scientific and Industrial Research (CSIR) in 1926. A full-time Director alone had complete authority for the management of the Institute. The comprehensive plan for research recommended by the Director, Sir George Knibbs, in 1921, could not be implemented for

lack of finance and again this attempt to form a permanent national institution failed.

When the government changed in 1923, Mr S. M. Bruce, later Lord Bruce, became the new Prime Minister. Bruce and his Ministers accepted the idea that Australia's development depended greatly on her ability to apply scientific methods to industry. The Prime Minister acted with speed and determination to alter and to improve the arrangements for this objective. In 1925 he personally presided over an authoritative conference of senior academic, industrial and political leaders from all States of the Commonwealth to discuss the reorganisation required. Sir Frank Heath, Secretary of the Department of Scientific and Industrial Research of Britain was invited to Australia and reported his views to the Government in January 1926.

Having decided that the Institute of Science and Industry Act (1920) must be amended, the Prime Minister invited George Julius, David Rivett and an engineer named Newbiggin to assist in drafting the Bill for an Act to found CSIR.

The Science and Industry Research Act was passed by the Parliament of the Commonwealth and given Royal Assent on June 21 and the Council had its first formal meeting on June 22–25, 1926.

When I went to Melbourne in 1945, just before my appointment to the Executive Committee of CSIR its members were Julius, Rivett and Richardson; Newbiggin had died in 1927 and Richardson had been appointed in his place. The determination and wisdom of these three carried this venture forward successfully for twenty years.

George Julius had distinguished himself as an engineer and at that time had a flourishing consulting practice in Sydney. He was an attractive and vigorous talker on all occasions. When chosen by the Prime Minister in 1926 to be the part-time Chairman, he was already known for his activities in the Institution of Engineers of Australia, through which he and others founded the Standards Association of Australia. He had a wide interest in many aspects of the economic life of his country.



Left to right: George Julius, Sir David Rivett, Dr A. E. V. Richardson.

*Sir Frederick White was Chairman of CSIRO from 1959 to 1970.

David Rivett had been persuaded to give up his Chair and his personal research in chemistry at Melbourne University to become the first full-time Chief Executive Officer of the new institution. His connection with the University of Oxford, where he went as a Rhodes Scholar from Melbourne, his friendship with Fellows of the Royal Society of London and with many eminent scientific men in England and Europe brought the CSIR into the environment of European science in the days when Australian scientists were few in number and lived at remote distances from that environment. David Rivett was a serious, kindly man who delighted in discussing with his scientific colleagues their work in the CSIR laboratories.

Richardson, always known by his initials A.E.V., joined the Executive Committee after being successively Superintendent of Agriculture in Victoria, Dean of Agriculture at Melbourne University, and the founding Director of the Waite Agricultural Research Institute in Adelaide. His profound knowledge of agriculture had a marked influence on the early programme of CSIR. Although, when I knew him, it was difficult for him to intervene in the flow of rhetoric of Julius, unless asked a specific question by Rivett, his deliberate statements were full of precise data and quite convincing.

CSIR consisted of the Executive Committee of three nominated by the Minister and appointed by the Governor-General, the Chairmen of the State Committees, and "such other members as the Council, with the consent of the Minister, co-opts by reason of their scientific knowledge."

The inclusion of the State Committees reflected the desire for a truly national activity, since acting through their Chairmen, the State Committees could refer to the Council problems considered important in their area. The co-option of additional members enabled the Council to provide itself with a wide variety of industrial, academic, agricultural or other advice.

It was not in the nature of Julius, Rivett or Richardson to do other than take the initiative; they did this with considerable success while deferring, as they formally had to do, to the Council.

Of prime importance to success was the fact that the Act placed in the hands of the Council the full authority to make such appointments to the staff on such terms and conditions as it felt necessary for the carrying out of the work to be undertaken subject only to the Minister's approval. The CSIR did not have to conform to the usual employment arrangements of the Commonwealth Public Service; the Executive Committee and the Council had a remarkable degree of freedom of choice of individuals and freedom to establish the conditions of employment that were thought most appropriate.

In the very early months two ideas were rejected. These were that the work of the new institution could be carried out under the guidance of specialist committees, and that the work itself could be undertaken in existing institutions, either State Departments of Agriculture or the universities. In the earliest discussions Julius took the initiative in rejecting the idea of committee control, holding that investigations undertaken in such a way would be protracted, that the committees would have difficulty in meeting, and would not be able to give the guidance and expedition to the activities that he desired. The Council agreed to appoint 'experts' to take control of related groups of investigations and passed a resolution "that in the opinion of this Council the problems awaiting solution are so vast and difficult that it is essential that men of outstanding quality should be secured to devote the whole of their time to efforts at solution".

Julius probably had some diplomatic difficulty in side-stepping the idea that the laboratories of other institutions could provide the venue for CSIR's investigations. Rivett, Richardson and the Council supported him and CSIR

avoided having to give way to pressures in this direction. Indeed one of the earliest decisions made was that the Council should establish its own laboratories. With regard to universities, the Council decided that it would be quite unsatisfactory to leave the important problems of the CSIR to be dealt with as the spare-time activities of the staffs of the universities.

Although a quarter of a century had passed since, by agreement with the States, the Australian Commonwealth was founded, the question of State-Commonwealth collaboration was still difficult particularly in specific areas. The founding of CSIR was not altogether welcomed by the State Departments of Agriculture which had been in existence since the very early days and which felt that they had a responsibility, which they were discharging effectively, to assist in the development and promotion of agriculture. This was important, particularly at that time, since the economic life of Australia was largely dependent on the agricultural and pastoral industries. Even Julius, the enthusiastic engineer, was one of the first to promote the idea that CSIR's early programme must be related mainly to the primary industries of the country. It was, therefore, essential that some agreement be reached with the States in the field of agricultural science.

The resolutions passed at a meeting between representatives of the Council and the State Departments of Agriculture, held in 1927, clearly outlined the basis for the division of research functions and did this in a way which set a tone for CSIR's future research. The conference confirmed the number and magnitude of the problems confronting the agricultural and livestock industries, it welcomed Commonwealth participation in research, and stressed that the field was almost unlimited in scope and that there were many problems, regional or national in range, and fundamental in character, which required concentration of effort and highly specialised research for their solution. The conference agreed that these problems were specially suited for investigation by the Commonwealth. In other words, the meeting handed over to CSIR, and I should imagine very much to Rivett's satisfaction, the more difficult long term problems that required scientific research in depth.

And so the Council began its task and must have done so with optimism for the future. In 1926, when the Act was passed, a sum of £250,000 was appropriated and placed in a Science and Investigation Trust Account for the purposes of the Council. In 1928 a further £250,000 was appropriated and placed in this account.

It is difficult today, so many years later, to envisage the Australia of that time. The main economic activities were rural, wheat and other crops, sheep, wool and beef cattle.

The first laboratories to be established were for the study of plant problems and entomology, in Canberra, animal health and nutrition in Melbourne and Adelaide and soils in Adelaide in association with the Waite Agricultural Research Institute. Studies of forest products grew out of early work in Perth but the main effort developed in Melbourne. A laboratory for fisheries investigation was established a few years later. The export of beef and lamb to the British market presented problems of cold storage which were to be studied, together with other problems of the food sciences, in laboratories in Sydney and Brisbane. The Australian Radio Research Board inspired by a similar arrangement in Britain, was set up and with a grant from CSIR and the Post Office began to support research in the universities.

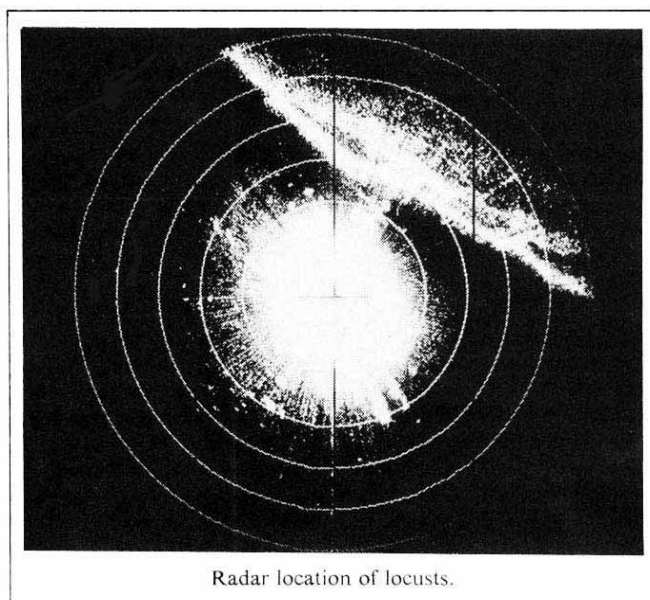
After these initial successes, the confidence of the Council was severely challenged when in 1930 the financial difficulties of the Commonwealth began to have an influence. The interest of the government was still strong but so serious was the financial position that the Trust Fund, already depleted, could not be adequately recouped. Survival depended on grants made to the Council by the

Empire Marketing Board, the Australian Pastoralists Research Trust and particularly the Rural Credits Development Fund of the Commonwealth Bank. To the relief of the Executive Committee, the Bank, in response to special pleading by Bruce in 1932-33, found £21,500 to make up the already severely reduced annual budget of £112,000. The Rural Credits Development Fund began making grants to CSIR in 1930 and continued to do so until 1939.

Having successfully ridden out the trials and tribulations of the depression years, the Council was prepared for the next great leap forward. The economic scene was changing with rural production being augmented by the production of the manufacturing industries. At the end of the First World War, the motor car was a curiosity but by the end of the 1930s, motor cars were common on the roads of Australia. As the depression years passed, a more prosperous community could take advantage of the general improvement in the quality and comfort of motor cars. Australian industry built the car bodies into which imported engines from the USA or Canada were fitted. This industry stimulated the manufacture of sheet steel, timber and leather products, paints, varnishes, as well as the manufacture of springs, bumper bars, shock absorbers, and so on. In the electrical field the use of what are today regarded as common appliances in the home was becoming more widespread. Firms such as Philips, Thompson Houston and the Australian General Electric, began manufacturing in Australia, or importing parts to be assembled here. Generators, transformers, telephones, and later refrigerators and domestic appliances generally were being made. Public broadcasting by radio began in 1920 and the manufacture of radio receivers began using imported parts; later the components were made by local industry. Likewise government expenditure on development was having its effect. There was a growing demand by public authorities for the construction of roads, irrigation dams and channels. The use of reinforced concrete was revolutionising building construction. Broken Hill Pty., and its associated companies, had begun steel production in Newcastle in 1915. By 1930, 25% of the output went as coiled rod to the wire industry for barbed wire, nails, fencing wire and netting. Much of the steel went into galvanised iron and a great deal of it was used to manufacture rails and other materials for the developing Commonwealth and State Railways. The scene was changing rapidly and very much in the direction of the interests of George Julius.

The Executive had been under pressure for some time to extend the activities of CSIR towards the interests of secondary industry. Julius, in 1936, saw in the government's request for research connected with the manufacture of engines for aircraft and cars the opportunity to advocate a wide ranging enquiry as to what the Council should do in the whole field of secondary industry research. The government appointed Julius chairman of a representative committee of members drawn from the engineering profession, industry, CSIR and the academic world to make a complete review of what was required. The recommendations of this Secondary Industry Testing and Research Committee, when adopted by the Government, transformed the activities of the Council.

The National Standards Laboratory was founded, and the beginnings of an Information Service approved. The setting up of the Aeronautical Research Laboratory and the Department of Aeronautics in the University of Sydney followed the special advice of H. E. Wimperis, Director of Scientific Research at the Air Ministry in the UK. At the same time the decision was taken to found the Division of Industrial Chemistry. When the war broke out in 1939 all these and some other activities connected with secondary industry were in the planning stage. By 1945, when the war ended, no less than 350 professional staff were employed in research related to secondary industry, a number



Radar location of locusts.

greater than had been employed in the whole of the activities of CSIR before the outbreak of the war.

Julius was in his element with the Division of Aeronautics and its major constructional undertakings for windtunnels and the like. This was a very successful venture but fell on hard times later when it had to be transferred from CSIR to the Department of Supply because of the rejection of secret work in CSIR. During the war, it was a flourishing institution, for the interest of the Royal Australian Air Force predominated. Rivett was happy with the development of the Division of Industrial Chemistry although there are indications that he had doubts about the rapidity of secondary industry research growth.

Professor J. P. V. Madsen, Professor of Electrical Engineering in the University of Sydney, had a special role in the founding of the National Standards Laboratory (NSL, now called the National Measurement Laboratory). Shortly after CSIRO began he made an approach to the Executive Committee advocating the need for primary metrological standards of physical quantities to aid industrial development. Although he chaired several committees his efforts were unsuccessful until supported by the government following recommendations by the Secondary Industry Committee. Madsen was given special authority by the Council to acquire a site to supervise the construction of a building and the allocation of the work of the different sections as they were formed.

The senior officers appointed to the National Standards Laboratory were in training in England at the National Physical Laboratory when the war broke out. They returned in time for the NSL to take part, with the Munitions Supply Laboratories, in providing a comprehensive measurement service to the munitions manufacturing industry. When the war ended NSL was able to take up its major role as the national laboratory for research on metrological standards and the measurement of physical quantities similar to the NPL in Britain and the National Bureau of Standards (NBS) in the USA.

Among the many men, members of the Council, industrialists or academics who had a role in the activities of CSIR, and later CSIRO, Madsen was outstanding among them. He graduated in engineering in Sydney, worked with W. H. Bragg in physics research in Adelaide and was appointed the first professor of electrical engineering in Australia: he devoted his life to the promotion of research in physics and engineering in the universities and in CSIR.

In 1939 the British Government invited Australia to

send a representative to England to learn of the secret development of radar. Acting on Madsen's advice, the Government and the Executive Committee selected Dr D. F. Martyn, an officer of the Radio Research Board for this mission. When Martyn returned, the Government agreed to set up a special radar laboratory in Sydney to play the part in Australia, although in a smaller way, played by Telecommunications Research Establishment (TRE) and other similar institutions in England. The Radiophysics Laboratory was founded and the staff grew during the war to 65 professional scientists.

It is questionable whether the activities recommended by the Secondary Industry Testing and Research Committee would have been accepted, let alone made such vigorous progress, if the war had not occurred. Because CSIR agreed to undertake important wartime work, money was readily available and growth was rapid.

When the war ended CSIR found itself with four major and several smaller institutions with programmes that had then to be changed to a peacetime civil basis.

Sir George Julius retired as part-time Chairman in 1945 and died in June of the next year, aged 73. David Rivett agreed to become the new part-time Chairman and Dr Ian Clunies-Ross and I, both appointed to the Executive Committee sometime earlier, continued with him as the new administration.

In retrospect it is clear how fortunate we were that David Rivett was the Chairman of CSIR when the political storm broke over us in 1948. The Liberal-Country Party, in opposition, attacked the Labour Government with accusations that the universities and CSIR were employing disloyal scientists with communist sympathies. It was alleged that important information from the USA was, as a result, being denied to Australia. Rivett was attacked personally when he advocated the return of CSIR to civil science and the setting up of separate defence research laboratories. The Opposition wanted CSIR brought more directly under the control of the Government. As is usual in these controversies, a great number of statements were made which were erroneous, irrelevant and of little real significance. The Prime Minister, then Mr Ben Chifley, and the Minister in charge of CSIR, Mr. John Dedman, took this matter very seriously. After due enquiry, the Cabinet decided that the Act under which CSIR worked had to be changed particularly to relieve the Minister of all personal responsibility for the appointment of staff.

It was Rivett's prestige, and the appreciation of Chifley and Dedman of what he and his colleagues had done for Australia that led the Government to insist only on essential changes in the Science and Industry Research Act. Indeed the new Act of 1949 placed greater responsibility on the 'Executive', of five members. The Executive would in future be responsible, with a few minor exceptions, for all appointments to the staff.

The new Advisory Council in this Act retained the same form as the old Council but had none of its administrative powers. With no governing Council the name had to be changed to the "Commonwealth Scientific and Industrial Research Organisation" (CSIRO). The "powers and functions" of CSIR were transferred unchanged to CSIRO and the scientific programme continued as before.

Rivett was, naturally, greatly distressed that the Government did not turn to the Council for advice. In retrospect, it is clear that the Government had to insist on certain changes to protect itself and the responsible Minister from false accusations. In spite of his disappointment and distress, Rivett, now nearly 64 years of age, who had reached the end of his formal period of appointment, consented to become the part-time Chairman of the new Advisory Council. He occupied this post for about a year and then became a part-time member of the Advisory Council for the following six years.

The proclamation of the new Act introduced a completely new regime. Ian Clunies-Ross was appointed the Chairman of the Executive, I was the Chief Executive Officer, and Dr S. H. Bastow, a physical chemist, was appointed as our third full-time scientific colleague. The two part-time members were Mr H. J. Goodes, an officer of the Treasury and Mr Don Mountjoy of Western Australia. Mr A. B. Richie, a grazier from the Western District of Victoria, replaced Mr Mountjoy in 1950.

The new administration entered into its responsibilities in a period of post-war euphoria. Money was easy to obtain and, indeed, the increase in our annual appropriation from the Commonwealth Government was quite large so growth, therefore, followed suit. We were able to introduce not only increases in the existing activity but some interesting innovations.

Pasture research, so successful in the south, was extended to the tropics using domesticated wild species of grasses and legumes from overseas. Animals more suited to the tropics than British breeds resulted from genetic studies of the beef-producing breeds. The physical and biological features of Northern Australia are now better known following extensive land-research surveys using novel methods. The unexpected widespread myxomatosis epizootic not only reduced the enormous rabbit plague to reasonable levels but was the opportunity for studies of the evolution of a virus in nature.

Money made available by the industry permitted a major expansion of research on the healthy sheep as a wool- and meat-producing animal. Wool textile research evolved new spinning methods and many novel treatments of wool textiles that have helped the International Wool Secretariat to promote wool in competition with synthetics.

Knowledge of the meteorology of the Australian sector of the Southern Hemisphere has been greatly enhanced since the beginning of early researches in meteorological physics. The National Measurement Laboratory is now a respected partner of the British NPL and the US NBS. Many researches of scientific and practical merit have over the post-war years emerged from what was originally the Division of Industrial Chemistry, now subdivided into several major independent laboratories; probably the most widely known is the development of the technique of atomic absorption spectroscopy.

The Radiophysics Laboratory, when work on radar ended, planned several peacetime researches. The curiosity of the late Dr J. L. Pawsey about extra-terrestrial radio noise took a major group of his colleagues into the new science of radioastronomy. By 1952, when the URSI met in Australia, no laboratory in the world could claim to have played a greater part in this new science.

The conquering of the pleuro-pneumonia epidemic in cattle, advances in cloud and rain physics and in the food sciences together with a major attack on the science of Australia's mineral resources are only a few examples of diversity of the research programme and its growth since 1945.

I have written perhaps too much of the Act, of the Executive and Council and said less than is adequate about the leaders of research whose imaginative concepts and scientific abilities made possible the achievements of CSIR-CSIRO. This is clearly inevitable in an account of how this institution started, survived the rigours of the depression and the war and escaped fatal change as a result of political expediency.

The early decision of the Council to appoint "men of outstanding quality" has been amply justified; it would be invidious and even tedious here to name and describe the work of the many men and women who have led the advance in pure and applied research. The record of their recognition by Australian and overseas learned societies and universities will speak for them.

Wildlife research

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The first work in CSIRO on wildlife was on the control of the rabbit, and this work still continues. But many other animals regarded as pests have also been investigated, from the Tasmanian waterhen to the kangaroo. Not all research has been devoted to pests, however, and recently the division has been pursuing an increasing amount of research on native fauna for its own sake.

WILDLIFE research began in the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in 1949. Before that there had been suggestions that the study of Australia's native fauna would be a desirable role for the Organisation but, in the economic atmosphere of those immediately post-war years and in the face of the enormous populations of rabbits that had developed due to the shortage of manpower and materials for control, the primary aim of CSIRO in forming the Wildlife Survey Section (later Division of Wildlife Research) was the study of the rabbit. Even so the founder, the late Dr F. N. Ratcliffe, saw beyond the immediate objective and anticipated that, ultimately, the work of the unit would include substantial programmes on native fauna.

The Australian vertebrate fauna is an important one. It includes most (119 species) of the world's marsupials, many bats and rodents, over 700 species of birds and over 390 species of reptiles. There are numerous endemic forms.

Research on the native fauna was certainly needed. The main era of inventory and of collection of specimens was over. There was some taxonomic research proceeding and there was an awakening interest in some State Authorities but, by 1949, there had been few programmes of research on the biology of native animals in the wild. This lack of data on the biology of the animals precluded effective management of them. Meanwhile agricultural and industrial developments continued to alter and to destroy the habitats and the populations of indigenous animals.

The Division of Wildlife Research has no direct responsibility to develop management plans for wildlife populations or to administer legislation about the protection or control of wildlife. In the various States of Australia and in the Australian Capital Territory and the Northern Territory these matters are the responsibility of local departments concerned with fauna conservation and the control of vertebrate pests. To ensure that the research findings of the Division are quickly made available to these departments, however, it maintains close contact with them, both informally and through liaison committees. This is a mutually helpful relationship since much of the Division's work has to be done in the field and depends on collaboration provided by the local departments.

The Division's major role in relation to vertebrate fauna is to provide information about the distribution, status, ethology, ecology and the population dynamics of these species. This provides a sound basis for the development of philosophies, strategies and tactics for management of the wildlife. The objectives of the management range from the preservation of rare species, such as the noisy scrub bird, *Atrichornis clamosus*, in the south-west of Western Australia, to the exploitation and harvesting of game animals, such as various species of duck, and to the control of populations of pest species, such as the European rabbit, *Oryctolagus cuniculus*.

The Division's field work is integrated with complementary studies of animals in experimentally-controlled laboratory and

enclosure conditions at the headquarters in Canberra, and at the Division's research centres in Perth, Darwin, and until recently, Alice Springs.

The Division's work on the rabbit is an example of its studies on a pest mammal. As this is the most important pest mammal in Australia it is fitting that a lot of attention has been given to it. The work has led to major practical advances in the control of the pest, and by clarifying our basic understanding of its biology and ecology has provided a substantial base for the development of more effective policies for control.

The initial plan for the study of the rabbit was for a long term wide ranging programme of investigation into the biology and ecology that might reveal 'chinks in its armour'. But a preliminary investigation of the distribution and status of the rabbit and of the damage it was causing in the agricultural and pastoral industries at the time indicated that there was then a more urgent need for shorter term studies of various control methods.

One of these methods was that of biological control through the use of myxoma virus which had already been investigated by the Division of Animal Health from 1936 to 1943 with generally discouraging results. In 1950 further field trials were started by the Wildlife Survey Section but this time they were in areas of higher rainfall. This led, late in 1950, to a mosquito-borne major epidemic spread of myxomatosis and, within a few months, rabbit populations over a wide area of south-eastern Australia had been drastically reduced by it¹.

For the next few years the Division studied in detail the disease and its vectors, its effect on rabbit populations, and changes occurring in the relationship between the virus and its rabbit host. This work was in collaboration with other divisions of CSIRO, and with the Australian National University, and with State departments involved in rabbit control. It became clear, as had been anticipated, that myxomatosis was not the panacea to the rabbit problem. Strains of virus, less virulent than the introduced strain and better adapted to survive in competition with the more virulent strains, had appeared in the field. There was also evidence of the development in the rabbit of a degree of inheritable resistance to the disease.

The Division investigated other methods of control, particularly that of poisoning, and as a result of screening tests showed that compound '1080' (sodium fluoroacetate) was potentially useful. Its use on a practical scale was then developed in collaboration with State departments and it became the main weapon of rabbit control on many thousands of holdings. Meanwhile the Division developed a series of studies of factors affecting the success of control operations which involve the use of poisons. This work led to recommendations about the technique of poisoning—about the type of bait material; about the placing of the bait; about the timing of the prefeeding before poisoning; and about the strategic timing of poisoning in relation to the time of the year when the rabbits were breeding. Possible side effects on non-target species were also being studied.

The Division then reverted to its original plan and, in 1961, began a series of long term studies of rabbit populations in a range of environmental conditions to determine the interrelationship of the various factors that affect the size of rabbit populations—weather, disease, predators. This has led to a better basis for integrating controls imposed by man with those occurring naturally.

The importance has been highlighted of the population remnants surviving after control efforts, and it is now clear



Birth of a Red Kangaroo. The new-born animal, 1.3 cm long, is about to enter the mother's pouch.

that in the past much control work was a poor investment because the degree of control was not high enough and little was done to prevent the residual populations from increasing. In some arid areas it has been demonstrated that definable survival areas—warrens in favourably situated sites—are of major importance. During a period of drought when rabbit populations are low or even nil over wide areas the survival areas harbour the nuclei from which populations may build up rapidly after drought. The strategic applications of control measures in these survival areas during droughts could lead to long term effective control over large areas.

These studies have also suggested that there is further scope for biological control of rabbit populations by introduction of internal parasites which do not occur at present in rabbit populations in Australia. The Division has recently begun studies of this possibility.

Several other animals have been considered pests of crops and pastures. Because of this, primary producers devote time and money to attempts at controlling them. State pest control authorities have not had the data to help them assess the true pest status of these species or to suggest management procedures. In the past the Division has paid a good deal of attention to these problem animals but now the need is declining and such work will form a less important part of the programme. The 'problem' animals that have been studied include several waterfowl, Anatidae; the Tasmanian waterhen, *Tribonyx ventralis*; wedge-tailed eagle, *Aquila audax*; galah, *Cacatua roseicapilla*; white-tailed black cockatoo, *Calyptorhynchus baudinii*; raven, *Corvus* spp.; emu, *Dromaius novae-hollandiae*; euro, *Macropus robustus*; red kangaroo, *Megaleia rufa*; grey kangaroo, *Macropus* spp.; and dingo, *Canis familiaris dingo*. In each case a substantial study of the life history has been made and at the same time the pest status of the animals has been established.

As one example the large kangaroos, *Megaleia rufa*, *Macropus robustus*, *M. fuliginosus* and *M. giganteus*, have been studied in the field and laboratory. The work has led to an understanding of population changes, movements, reproduction, food and growth and the effects of environmental influences on them. A secure base of data is now available on which management plans can be based. These animals are subject to pest control operations and, more recently to, a high degree of commercial harvesting and doubts have been expressed as to the future of the species.

The Division now believes the animals can well withstand harvesting provided management plans are based on the biological data that are now available.

Of particular significance was the finding that populations of some (if not all) species of large kangaroos, flourish on the disclimax plant communities that are produced by domestic

stock grazing on native habitats. In severe drought, stock and kangaroos do compete for food but in most years the competition is not great because the two groups of animals tend to select different food items. It is suggested that much land would have greater productivity if both stock and kangaroos were grazed, for harvesting, than if it were devoted to grazing by only stock or kangaroos.

In these studies of 'problem' animals basic biological data accrue, for example, reproduction processes in marsupials, social structure of canids, physiological adaptation to arid zones by euros. In addition, there are other, unexpected results. The study of kangaroos led to the discovery of a previously unrecognised second species of grey kangaroo, *M. fuliginosus*. The discovery was based on observed differences in blood proteins and in morphology, physiology and behaviour. In the study of ravens a fourth species, *C. mellori*, was identified on the basis of behaviour and later shown to have distinctive plumage, movements and food habits. In the white-tailed black cockatoo, two forms (perhaps species) have been shown to exist; they differ in skull and bill characteristics, distribution and ecology. In addition to the intrinsic biological value of these discoveries they have significant implications in management.

The range of climates in Australia and, in particular, the extensive arid and semi-arid zone has encouraged considerable attention to be given to the study of the factors controlling the breeding of birds. Particular attention has been paid to water-birds.

In the regulation of breeding the importance of environmental factors has been emphasised. Among the inland waterfowl (Anatidae) some species, for example, *Biziura lobata* and *Oxyura australis*, have regular sexual cycles that are probably controlled by photoperiod, but in two, *Malacorhynchus membranaceus* and *Anas gibberifrons*, it has not been possible to show any regular cycles and breeding is controlled mainly by environmental factors. In some species such as *Anas superciliosa* and *Aythya australis* the photoperiodic effect exists but the cycle is strongly affected by environmental factors.

In the first group regular breeding is normal, in the second group the breeding season is quite erratic and can occur at any time of the year when favourable conditions occur. In the third group some birds breed in all years at a reasonably well fixed period but when favourable conditions occur extensive out of season breeding follows. The principal environmental factor affecting the sexual cycle is rising water level and flooding that



The Mallee Fowl, *Leipoa ocellata*, an Australian inland bird that does not brood its eggs but buries them in mounds of sand and vegetation.

can occur in locally dry conditions due to rain on distant catchments.

The work on reproduction is supported by studies of food habits, behaviour and movements. The demonstration of the erratic breeding seasons and the demonstration of widespread nomadic movements and the explanation of their causes help State fauna conservation authorities to devise appropriate management procedures.

The Division now believes that its principal contribution to the biological base of waterfowl management is concluding and is moving emphasis to the granivorous dry land birds of the arid and semi-arid zone. The breeding of all arid zone pigeons and the stubble quail, *Coturnix pectoralis*, are under study. Preliminary results show that all of the arid zone pigeons and the quail have regular annual sexual cycles but, with few exceptions, there are strong effects of rainfall on those cycles and on the breeding seasons. There is reason to believe that the rainfall operates through the availability of food and hence the nutrition of the birds. A similar explanation was suggested for the effect of changes in water-level on the cycles of waterbirds.

In the well-watered tropical north although the breeding seasons of waterbirds are, superficially, regular they are to a large extent controlled by changes in water level, as in the arid zone. In some species, for example magpie goose, *Anseranas semipalmata*, the effect of water level that is important seems to be the density of swamp vegetation available for nesting. Granivorous pigeons of the tropics, as in the arid zone, have breeding seasons largely controlled by rainfall and its effects on food supply.

Management of populations of many animals is not possible without precise data on their movements and other biological attributes that can only be collected from individually marked animals in the wild. For that reason, in 1953 the Division set up the Australian Bird-Banding Scheme and in 1961 the Australian Bat-Banding Scheme. These are operated as services. In addition to the Division's own staff, university staff, State authorities and amateurs can become registered banders on approval of their proposed projects and their competence to band. Bands, other equipment, office support and advice are provided; copies of all data are kept in the Scheme's archives. In 1975 there were 338 registered banders. Since 1953 1,270,000 birds of 780 species have been banded in Australia and its Territories and 270 papers have been published utilising results of the banding scheme. In the more specialised Bat-Banding Scheme there are now 12 registered banders and 65,000 bats have been banded.

Some elements of the Australian fauna, particularly the ground-living mammals had declined before settlement and the effects of introduced grazing animals and predators. In vast areas of the continent there is no real idea of the present status of many forms. For these reasons a survey group was established in 1962.

Regions for surveys are selected because of the imminence of development or because of their intrinsic faunal interest. They are visited by parties comprising ornithologists, mammalogists, herpetologists and botanists. Each region is visited several times at different seasons. Data on the distribution, present status, habitat needs and other aspects of the biology of many species are collected. The planning of conservation reserves and their ultimate management can proceed on the basis of these data, and conflicts with mining and other developments can be viewed in better perspective.

The survey group has worked in New Guinea and continues to work in southern Australia but now believes that its main role is in the sparsely settled north of the continent and is concentrating on Arnhem Land at present. In later years it is planned to expand both east and west from that area.

In addition to what might be called the predictable results many surveys produce novelties; animals considered very rare or extinct are discovered in some numbers (for example, the pygmy possum, *Burramys parvus*; the delicate mouse, *Leggadina delicatula*; the Australian native mouse, *Pseudomys, novae-hollandiae*; the marsupial mice, *Antechinus bilarni* and *A.*



An Echidna, *Tachyglossus aculeatus*, just hatching from its egg. The Echidna and Platypus, both monotremes, are only found in Australia.

bellus; the rock rat, *Zygomys woodwardi*; and the Kangaroo *Macropus bernardus*). Nests and eggs of some birds are described for the first time (for example, *Ptilinopus alligator*, *Petrophassa rufipennis*, *Amytornis woodwardi*) and the known ranges of many animals are greatly extended.

In addition to the formal survey and censusing of wildlife in various regions the Division is planning to develop methods of rapid survey and evaluation of fauna in the whole continent. Work has begun, in conjunction with other Divisions, to establish methods to classify and to rapidly assess the value to aquatic fauna of the wetlands of the continent. It is hoped to begin soon to do similar work with dryland habitats and so provide the faunal component to the long term Ecological Survey of Australia that is under discussion.

Much of the work of the Division to date has been concerned with single species or with groups of species of animals. The Division has been aware for some years that major advances could be made by the examination of whole communities and it plans to develop this work more in the future. The first such study became possible when a laboratory was constructed in Darwin, Northern Territory in 1971 and a group of mammalogists, ornithologists and botanists was established there. The aim is to study the productivity and management of tropical grasslands and their fauna, particularly as these are affected by the introduced water buffalo, *Bubalus bubalis*, and feral pig, *Sus scrofa*.

The group now has been given control of a large study area wherein field laboratories are to be established, on the South Alligator River. In the first phase of the group's work the ecology of some of the more important animals and habitats has been studied. The next phase is now beginning and the work of each person will be closely integrated into the main theme. Considerable opportunities will exist for visiting scientists to participate in this programme.

Most of the work of the Division has been ecological or behavioural and studies of physiology have been used mainly in a supporting role although there have been exceptions, such as fundamental physiological, anatomical and biochemical research on the echidna, *Tachyglossus aculeatus*, and on the platypus *Ornithorhynchus anatinus*, reproduction in kangaroos and olfactory communication in rabbits. Recently, in line with the Division's increasing role in research on the native fauna for its own sake, a physiological group has been established that will undertake long term research on the fundamental physiology of marsupials.

The future trends in wildlife research in CSIRO can be summarised as increasing attention to research by multi-disciplinary groups on communities rather than on groups of species or single species and increasing attention to research, including surveys of fauna in remote regions, particularly in tropical Australia.

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Animal nutrition to human nutrition: achievements and prospects

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The Division started in 1927 as one of Animal Nutrition, particularly interested in the role of trace elements in sheep. Progressive changes in title of the division clearly show the way emphases have changed, until now, although there is still much animal work being done, new programmes are being planned with particular relevance to human welfare. Some of this work will be in biochemistry and physiology, but the division is also committed to ecological and behavioural approaches to human nutrition.

IN 1927 the Council for Scientific and Industrial Research (CSIR) established a laboratory in South Australia at the University of Adelaide with the intention that it be involved in research into animal nutrition with particular relevance to problems concerning the agricultural and livestock industry of Australia.

During the ensuing years this laboratory, first as the Division of Animal Nutrition (1927–33), then as part of the Division of Animal Health and Nutrition (1934–44) subsequently as the Division of Biochemistry and General Nutrition (1944–65) and, finally, the Division of Nutritional Biochemistry (1965–1975), became well known for its contribution to various aspects of ruminant metabolism, with particular emphasis on the role of trace elements. Successive Chiefs were Professor T. Brailsford Robertson (1927–30), Sir Charles Martin (1931–33), Dr L. B. Bull (1935–44), Dr H. R. Marston (1944–65) and Dr A. T. Dick (1965–75).

When, in 1975, a decision was made to establish a research centre in Australia for work on human nutrition within the structure of CSIRO it was opportune to redirect the activities of the Division of Nutritional Biochemistry and incorporate much of the expertise and experience built up over many years of research in animal nutrition into a new Division of Human Nutrition.

Since Dr B. S. Hetzel, formerly Professor of Social and Preventive Medicine, Monash University, was appointed Chief, the Division of Human Nutrition has begun to develop research programmes with particular relevance to human welfare. Some of these are a natural extension of areas covered in earlier work with sheep. The background of past achievements provides a challenge for the future of the new Division. Initially three main programmes in human nutrition are planned—trace elements and mineral metabolism, metabolism and digestion, epidemiology and human ecology. The achievements of the past and the prospects of the future will now be reviewed.

Research in trace elements

A major concern of the Division for more than 40 years has been the role of trace elements in the nutrition of sheep. This interest stemmed from the realisation that animal husbandry in extensive, well-watered coastal tracts in the comparatively arid state of South Australia had been rendered precarious from the earliest days of settlement by the uncertainty of pasture and crop production and the risk of Coast Disease in

grazing sheep and cattle. Sheep restricted to these regions commonly developed a profound anaemia, lost appetite progressively and died in an emaciated condition within a matter of months.

In 1928 Sir Arnold Theiler, then Director of the Onderstepoort Veterinary Research Institute had been invited to visit Australia and advise the newly established Council on profitable avenues of animal research. He suggested that Coast Disease in sheep, in which bone fragility was a common feature, might well be due to phosphate deficiency. This hypothesis was disproved experimentally and, in the early thirties a Coast, Disease Committee was formed by Sir Charles Martin to coordinate the systematic study of the disease.

The highly calcareous affected soils were composed almost exclusively of comminuted shell fragments blown from the continental shelf exposed during the last Ice Age and it was predicted that they would lack not only phosphate but also traces of heavy metals. Some of these (for example, iron and copper) were already known to be essential animal nutrients.

Sheep offered a lick containing small amounts of seven metallic elements, including cobalt, showed a limited response and on the basis of a German report that cobalt could induce polycythemia in rats, the possibility that cobalt deficiency might be intimately associated with the anaemia and other features of Coast Disease was explored. Pure cobalt nitrate given at the rate of 1 mg Co/d elicited dramatic responses in coastal sheep at pasture or in pens (Fig. 1). Subsequently, copper deficiency was shown to complicate the primary deficiency of cobalt. An historic account of these events appeared in 1938 (ref. 1).

Cobalt proved to be effective only within the rumen² but the cumbersome necessity to provide cobalt supplement by frequent drenching or other means was eventually overcome by exploiting a suggestion by a visiting Fulbright Research Fellow, Professor Perry R. Stout, that a device might be lodged in the sheep's rumen to release cobalt slowly and continuously. A heavy pellet, comprising cobaltic oxide and iron powder, was evolved

Fig. 1 Merino ewes depastured on grossly deficient shell-sand dunes at Robe, South Australia. The emaciated ewe (left) has been given copper but is suffering the final stages of cobalt deficiency; the comparable healthy animal (right) has been adequately dosed with both cobalt and copper.

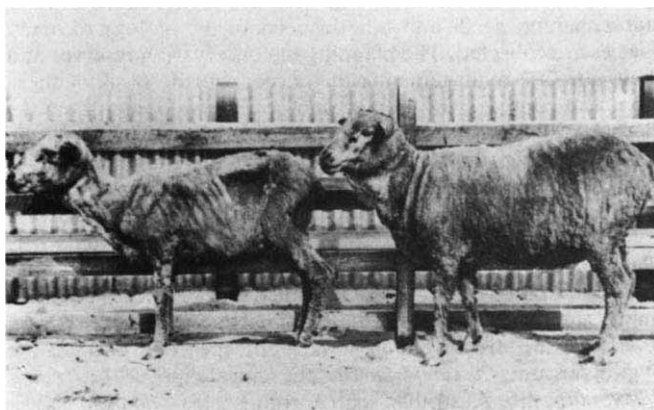




Fig. 2 Effect of copper deficiency on Merino wool⁸. The copper-deficiency lesion is apparent in the upper part of the staple grown by an acutely deficient ewe. Restoration of adequate copper status resulted promptly in normal crimp in later growth.

in the Division which, given with a steel grinder to reduce a tendency for calcium phosphate to encrust the pellet, satisfies these requirements for Merino sheep in most circumstances³.

In sheep maintained by cobalt supplements, the effects of copper on a specific deficiency lesion in wool (Fig. 2), on wool growth, fleece pigmentation, anaemia and growth were studied intensively⁴ and led, early, to the application of copper fertilisers with spectacular results⁵ to crop and pasture species which had invariably failed on these recalcitrant soils (Fig. 3). The successful development of large tracts of soils derived from the calcareous sands was made possible. Cereal crops, improved pastures and healthy flocks flourish where only stunted scrub had previously grown, in spite of an adequate rainfall.

Space does not permit reference to studies with selenium⁶ and other minor and major elements except to mention that the principles involved in the useful development of the cobalt pellet have been applied to the production of a selenium pellet⁷ which provides a ewe and her suckling lambs with their selenium requirements for several years and so prevents the occurrence of White Muscle Disease in lambs in deficient areas.

Many aspects of these researches, which changed the face of agriculture in South Australia and have been widely applied elsewhere, have been reviewed by Marston^{8,9}.

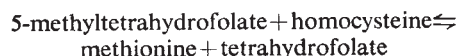
In 1948 came the recognition^{10,11} that vitamin B₁₂, the newly isolated anti-pernicious anaemia factor, contained cobalt. Within a short time workers at Cornell¹² had shown that cobalt-deficient sheep responded to injected vitamin B₁₂. This finding was quickly confirmed in the Adelaide laboratory¹³ and it became apparent that the sole function of cobalt in the

rumen was to enable the microflora to synthesise vitamin B₁₂ (ref. 14). There followed a series of metabolic^{15,16} and biochemical^{17,18} studies into the nature of the disease.

The most prominent effects in sheep are depression of appetite, inefficient use of food and severe depletion of the stores of folic acid in the liver. Most of these effects can be attributed to depressed activity of two vitamin B₁₂-containing enzymes. The first of these¹⁷ is methylmalonyl-coenzyme A (CoA) mutase which catalyses the reaction



one of five sequential steps in the conversion of propionate into succinate. Ruminants derive a substantial part of their energy from propionate produced in the rumen and its accumulation in the systemic blood appears to produce the loss of appetite so characteristic of the disease¹⁶. The second critically affected enzyme¹⁸ is the methyl-transferase that catalyses the reaction



Failure of this reaction to occur leads to a deficiency of available methionine. The lack of methionine is responsible for the appearance of fatty liver and may limit body growth. It is also responsible for the depletion of liver folates¹⁹.

The appearance of megaloblasts in human pernicious anaemias is generally attributed to impaired synthesis of DNA due to a lack of 5, 10-methylenetetrahydrofolate. The severe depletion of folate stores that occurs in vitamin B₁₂-deficient sheep is not accompanied by delayed maturation of red cells, however, and further work to study this aspect of human pernicious anaemia will be carried out in the monkey.

Recent work in the Division on copper deficiency has been directed at the disease of young lambs known as enzootic ataxia or swayback. The condition is characterised by degeneration of myelin in the spinal cord and may be prevented by dosing the pregnant ewe with copper. Current work is concerned with the biochemical mechanism by which copper exerts its effect on development of the central nervous system of the foetal lamb. These studies may prove to be relevant to the degenerative brain disease of children known as Menkes' kinky hair syndrome²⁰ which has been traced to a systemic deficiency of copper.

The multidisciplinary approach to the resolution of trace element deficiency diseases in animals may be appropriately directed to problems in human nutrition. Epidemiological

Fig. 3 Copper response in wheat⁵. Wheat grown on calcareous sand at Robe, South Australia. Foreground, superphosphate only; background, superphosphate plus copper sulphate.



studies precede the recognition of a nutritional deficiency and define the problem as with Coast Disease. An intensive study of physiological or microbiological implications follows and, finally, as in the case of cobalt, definition of the precise function of the missing nutrient is reached in biochemical terms.

One of the most widespread and certainly the most obvious of trace element deficiencies in man is that of iodine, and work related to iodine deficiency in the human population will form one of the major new studies in the reconstituted Division. Extensive epidemiological work has already been done^{21,22}.

Endemic goitre and the associated condition of mental deficiency, deaf-mutism and spastic diplegia known as endemic cretinism has been recognised in Europe, and especially in the Alps, for centuries.

Studies in New Guinea²³ and elsewhere revealed that endemic cretinism (Fig. 4) was associated with a more severe iodine deficiency than was endemic goitre. Iodine intake was usually less than $20 \mu\text{g d}^{-1}$ as compared with a normal intake of about $100 \mu\text{g d}^{-1}$.

A long standing controversy as to whether endemic cretinism was related specifically to dietary iodine deficiency led Hetzel, Butfield and Pharoah to set up a controlled clinical trial in the highlands of New Guinea to determine the effectiveness of a single injection of iodised oil as a long term prophylactic. By 1970 it was apparent that effective prevention had been achieved provided the mother had received an injection of iodised oil before, and not during, pregnancy²⁴. Extensive mass injection campaigns have now been planned and carried out in a number of countries including New Guinea, Nepal, Peru, Ecuador and Zaire.

From the results of the trial, iodine seems to have exercised a specific effect on the early development of the central nervous system, perhaps during the phase of early neuroblast multiplication from 10–18 weeks²⁵. This phase of brain growth predates the achievement of full competence by the foetal thyroid gland and raises the question of whether thyroid hormone or iodine itself may be responsible for protection against the disease²⁴. Experimental work to study the mechanisms involved at the physiological and biochemical levels is being actively pursued in the new Division, using first the sheep and later the monkey as the experimental model.

Metabolic studies

During the period 1939–46 research into the digestive processes within the rumen of sheep by a group at Cambridge²⁶ and by Marston²⁷ and Gray²⁸ at the CSIR Animal Nutrition Laboratory established the importance of microbial degradation of fodder to short-chain fatty acids in the rumen.

The three main fatty acids (acetic, propionic and butyric) produced in the rumen and absorbed from this forestomach were shown to provide much of the energy requirements of sheep. It was also established that ruminants, unlike monogastric animals, absorb virtually no glucose as such from the alimentary tract. They rely on gluconeogenic processes in the liver for their supply of glucose by conversion of propionate absorbed from the rumen, and of amino acids derived from microbial protein and absorbed from the intestine.

This work established the pattern for much of the subsequent research in animal nutrition and demonstrated the relevance of basic research to such agricultural problems as drought feeding, efficiency of utilisation of pasture and pregnancy toxemia.

While one group^{29,30} was elucidating the effects of dietary factors on the production and relative proportions of short-chain fatty acids in the rumen, another group^{31–33} was studying the intermediate processes involved in the utilisation of acetate for energy requirements and of gluconeogenic precursors for glucose production.

During this period (1950–65) many of the questions being asked about the efficiency of utilisation of different types of fodder were being resolved by microbiological and physio-

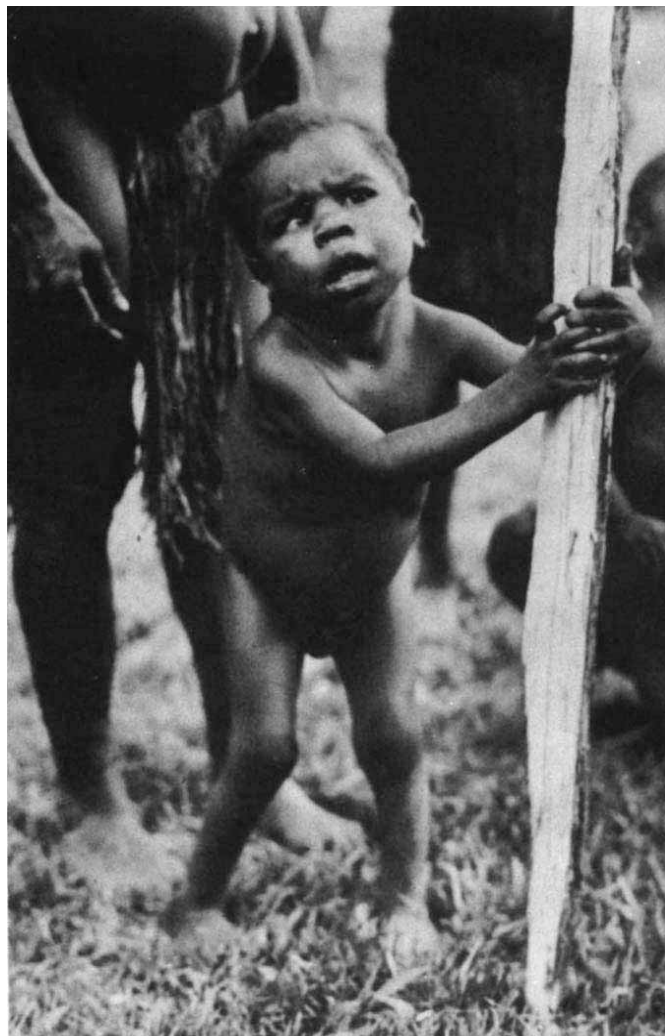


Fig. 4 A young New Guinea boy with cretinism, showing squint, vacant facies and leg deformities²¹. Permission has been granted by the editors of *The Medical Journal of Australia* for the publication of this photograph.

logical attacks on the problem. There was, also, a growing interest in enzyme mechanisms and the control of metabolic pathways in ruminants³⁴. This work, while continuing to lay down foundations for further understanding of ruminant nutrition, led to a more acute awareness of biochemical differences between ruminants and monogastric animals, particularly in relation to the significance of gluconeogenic and lipogenic pathways.

It was shown elsewhere³⁵ that ruminants lack two important enzymes involved in a lipogenic pathway from glucose in liver and adipose tissue. Hence sheep normally rely on acetate as a lipogenic precursor rather than on glucose which is the major source for fat synthesis in monogastric animals.

Later studies in the Division proved that these two enzymes are adaptive³⁶. In newborn lambs reared on a high carbohydrate diet or in adult sheep receiving large amounts of glucose administered intravenously or into the abomasum (and thus by-passing the rumen) these enzymes are present. Glucose can then act as a lipogenic precursor and in this respect sheep behave metabolically like monogastric animals.

The expertise acquired in animal nutrition is now being applied to the three new metabolic programmes envisaged for the Division—digestion and gastrointestinal disorders; efficiency of utilisation of food stuffs and metabolic aspects of obesity; protein metabolism, degradation and turnover.

With the growing concern about the preference for highly

refined foods in Western society and the epidemiological correlations reported for related gastrointestinal disorders, an experimental study will be made of the effects of dietary fibre in monogastric animals. Various components of fibre, their relationship to water absorption, bile salt degradation and rate of passage of digesta will be examined. A collaborative programme with a Clinical Gastroenterology Unit is being planned.

Fundamental studies on protein degradation using isolated cell preparations have been actively pursued³⁷. Measurements with cultured cells were commenced to establish a model system in which various hormones, nutrients and other compounds could be tested for their effects on general protein degradation and on proteolytic pathways for the removal of error proteins. These studies will be applied to problems relevant to wasting diseases, growth and ageing.

Work currently being undertaken with isolated hormonally responsive rat liver cells on interrelationships between high carbohydrate and high fat diets, metabolic pathways and hormone efficacy will be integrated with whole animal studies on metabolic and hormonal aspects of obesity.

Human nutrition in an ecosystem

In its pursuit of solutions to problems in human nutrition, the Division will be taking a further conceptual step—denoted by the concept of the ecosystem. The application of this concept will now be considered in relation to two urgent nutritional problems in Australian society—the nutritional state of the Australian Aborigines and the problem of alcohol consumption.

An ecological approach is urgently required for a distinctively Australian problem in human nutrition—the nutrition of the Aborigines. Gross malnutrition, particularly of infants, is very common among the Aboriginal people. It is a major cause of the unacceptably high infant death rate in the first year. Compared with an infant mortality of 20.7 per 1,000 live births for white Australians in 1967–69, Aboriginal infant mortality in 1965–69 averaged 112 for the Northern Territory³⁸. This gross malnutrition has resulted from complete disruption of the normal pattern of living which represented a reasonably satisfactory adaptation before the arrival of the white man^{39,40}. Loss of tribal lands and contact with white Australian society has been accompanied by cultural disintegration leading to loss of motivation for hunting and the tribal way of life. One by-product of this is malnutrition in infancy, another is alcoholism in adults. Improvement may come from an approach designed to develop a viable ecosystem, as independent of white society as possible, to give the Aborigine time and opportunity to reintegrate with his environment.

There is a challenge to Australia to do something much more imaginative for the Aborigine than has been done so far. CSIRO has much to offer here, concerned as it is with new approaches to energy and water conservation, soil vegetation and feral animals, as well as nutrition and housing.

Alcohol consumption is rising progressively in Australia as in other developed industrial urban nations. Surveys of levels of consumption reveal 6.5% in excess of an average of 100 ml d⁻¹ of absolute alcohol in 1971 compared with 3.4% in excess of this level in 1949. The level of 100 ml d⁻¹ is generally considered a high risk level for a traffic accident, serious physical disease and death before the age of 50 yr. Emphasis is being laid on levels of alcohol consumption rather than on "alcoholism" because of the need to intervene much earlier in the natural history of the disorder to have some chance of successful treatment and rehabilitation. An elevated blood alcohol is found in more than half of the victims of fatal traffic accidents and the numbers are increasing each year. Excessive alcohol consumption is a significant factor in at least 20% of hospital admissions and a major factor in family breakdown and serious crime.

Alcohol consumption is increasing most in the young adult group, especially in young men. This phenomenon is clearly related to the social environment—the high social approval of

heavy drinking, the promotion of drinking in association with sport and a masculine out-of-doors image. The lack of adequate recreation and education for leisure in an affluent society accentuates the problem. There is an urgent need for research to understand the genesis and maintenance of the life style of the young adult with a view to development of a suitable social strategy to reduce alcohol consumption⁴¹. An ecological approach with special attention to family, school, peer group and mass media is clearly indicated. Education, legislation and new services will all have a place in any such social strategy. Furthermore, alcohol intake should not be isolated from other aspects of diet such as carbohydrate and fat intake or life style including cigarette smoking, working and leisure patterns.

Conclusion

In considering this transition of a Division of Animal Nutrition into a Division of Human Nutrition, we have to recognise that much more is known about the basis of animal nutrition because of the better opportunity for experimentation. Human nutrition needs to rely on animal models for final clarification of mechanisms. The application of chemical, biological and behavioural approaches depends however on adequate epidemiological study in the first place. Once this definition has been achieved, means will need to be devised for appropriate modification of human dietary patterns—a difficult task as indicated by existing problems in modifying cigarette consumption in spite of overwhelming evidence of relevance to lung cancer.

The Division is therefore committed to an ecological and behavioural approach to human nutrition along with the more traditional disciplines of biochemistry and physiology. Only with a total view of man can adequate understanding be achieved.

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Growth of CSIRO's interests in food research

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In 50 years CSIRO has developed its interests in food research from a few projects on urgent technical problems confronting the export trade to an organisation with three large laboratories engaged in broadly-based programmes of basic and applied science.

ON the second day of its first meeting (April 14, 1926), the Executive of the Council for Scientific and Industrial Research (CSIR, later the Commonwealth Scientific and Industrial Research Organisation, CSIRO) drew up a list of the "urgent and promising" lines of investigation on which it considered immediate scientific effort must be concentrated. One of the five subjects listed was food investigations; after some discussion the members of the Executive divided this into research on the problems of dairy manufacture and research into food preservation, with particular attention to problems of cold storage. The need of industry for such scientific and technical help had been uncovered by failures in Australia's important export trade in foodstuffs, which was worth \$A25 million in 1926 and is today worth more than \$A1,000 million. "Industry that relies solely on the scientific work of other countries", so the Executive judged, "cannot hope to occupy a commanding position in the world market". Within Australia too, the normal problems of transport and long term storage of perishable foods were exaggerated by the tremendous distances and the uneven distribution of population. CSIR's interest, however, was only incidentally in industry's housekeeping problems; its primary aim was to increase the sum of knowledge on food and food constituents. Research was to be of a fundamental nature, and on problems that transcended state boundaries and required the long term attention of a team of specialists.

From such beginnings grew one of the largest organisations in CSIRO. The present Division of Food Research has some 350 people working for it, of whom about 40% are professional scientists; the Australian Government provides about 80% of its budget, exceeding \$A4 million a year, with most of the remainder contributed by the meat and dairy industries. This Division, which resulted from the amalgamation of the Division of Food Preservation and the Division of Dairy Research, has become the major Australian centre for research studies in food science and technology. It has large research laboratories in four states, each specialising in work on particular foods such as fruit and vegetables, poultry and eggs (New South Wales), meat (Queensland), dairy products (Victoria), and seafoods and potatoes (Tasmania). CSIRO also makes grants to the two trade-directed Institutes of Bread Research and Wine Research, and a few food investigations are carried on in other divisions such as Entomology (storage of bread grain) and Horticultural Research (disorders of fruits).

For an integrated approach to the problems of food science it is necessary to employ workers in many disciplines, such as physics, chemistry, biochemistry, microbiology, engineering and the physiology of plants and animals. Such highly trained scientists, particularly any with special qualifications for food research, were not numerous in Australia during the 1920s. CSIR began by selecting likely-looking leaders from among the young university graduates and sending them to England on scholarships; there they obtained specialist training at the Low Temperature Research Station, Cambridge, or at the National Institute of Dairy Research. The resulting delay in setting up

any CSIR organisation for food research could scarcely have been avoided; nonetheless, it had serious consequences. When the first trainees, J. R. Vickery and W. J. Wiley, returned to Australia in 1931 the depression had already hit the country and CSIR was struggling to keep going. Establishment of two new divisions, or of any, was out of the question. Virtually all dairy research was postponed indefinitely and work on food preservation was confined to the two commodities of greatest importance to export—beef and fruit—and had to be carried out in laboratories lent or shared by other organisations. The Section of Food Preservation (a Division from 1940) was established in 1931 with Vickery as leader and seven other research officers. The Section of Dairy Research (Division, 1962) under Wiley could not be formed until 1940.

Depression and the Second World War

The new group's first resounding success came with its first undertaking: it played a central role in initiating the export of chilled beef to Britain, thereby opening a lucrative market to the Australian cattle industry^{1,2}. This is probably the longest lived of all the Division's lines of investigation: research on chilled beef is now important for the new packaging and quality requirements of the Japanese and US markets. CSIR had been able to start work on fruit storage and ripening as early as 1927, in collaboration with the State Departments of Agriculture, and the Division's thoroughgoing investigations in this area made it possible to develop a scientific basis for the storage and export of pears, apples and oranges, and to recommend revolutionary new methods for transport and ripening of bananas within Australia.

When the country finally emerged from the Depression in the late 1930s, new and more central laboratories were set up at Homebush, New South Wales, but plans to establish food research on a more comprehensive basis, embracing basic food science studies as well as empirical work on all the important commodities, were foiled by the outbreak of the Second World War.

The war made exceptional demands on Australia's food-growing and food-processing industries, and the Division of Food Preservation together with the embryo Division of Dairy Research in Melbourne were the only centres in Australia where research on food technology was being carried out and where there was a group of people who could advise the Government on problems in this field. Now attention moved away from problems of cold storage to concentrate on methods of preservation that did not require refrigeration, such as artificial skin-coating of fruits, dehydration and, above all, canning. Although Australia had exported canned meat for 75 years, knowledge of the canning of vegetables and fruit juices was sadly lacking. Yet Australia was required to supply a wide range of preserved foods for the armed forces of the USA and Australia in the South-west Pacific area. The technology needed to bring the Australian canning industry into the twentieth century was worked out in the Homebush laboratories, with the willing cooperation of visiting American food technologists and of industry leaders. Similarly, the Division of Food Preservation developed and prepared the first survival ration packs of concentrated foods for the Australian Army. The Division of Dairy Research created a butter with properties suitable for use in the tropical theatres of war. Both divisions acted as service laboratories for the Government, vetting specifications, carrying out regular quality control checks, issuing certificates—all jobs

that were anathema to the original concept of CSIR and that were shed as soon as possible.

Food preservation after the war

The staff of the Division of Food Preservation had doubled during the war and the expansion of staff and resources continued. It was now essential to devote more attention to fundamental work to obtain a better understanding of the constituents of foods and their reactions to different environments, and so provide a basis for further advances in the applied field. The division was reorganised so as to devote about 50% of all its resources to food science. Three groups were formed at Homebush, in chemistry, physics and microbiology; there was no room for more in what had become extremely cramped laboratories. By an imaginative pooling of resources with the University of Sydney, which lacked senior research staff as much as the Division lacked space, it was possible to form two new units—the first in Australia—of plant physiology and physical biochemistry, which were located at the university.

All these groups carried out important basic research which earned the Division an excellent international reputation.

The research workers in the Plant Physiology Unit, for example, made notable biochemical and biophysical contributions to the knowledge of the mechanisms of the active transport of cell constituents against osmotic gradients³. They greatly increased the understanding of the ripening of apples and bananas, particularly in relation to the effects of ethylene⁴.

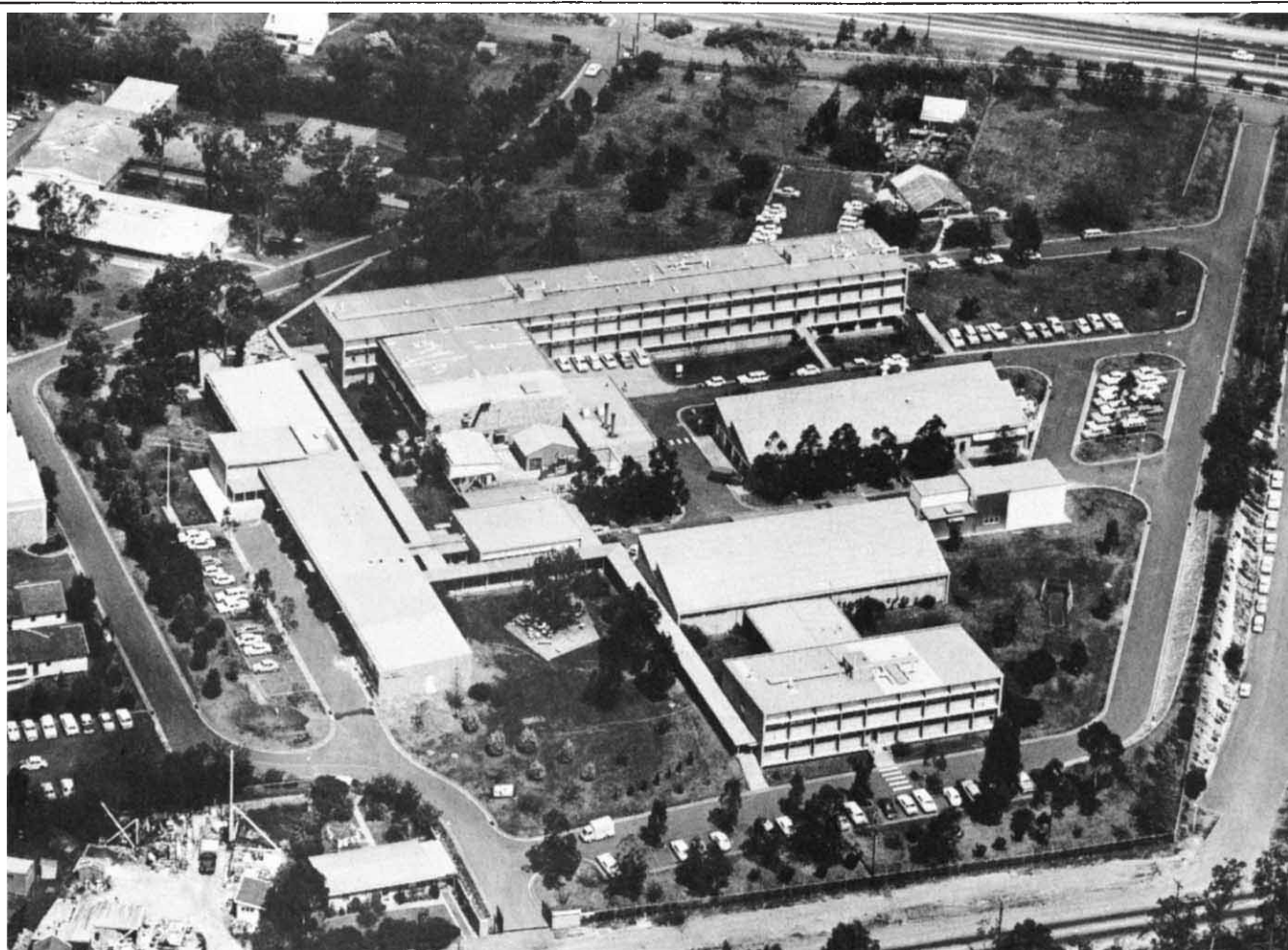
Work by the Physics Section on the evaluation of heat penetration processes in relation to the survival of heat-resistant bacterial spores during canning is one of the classics of food technological research⁵.

The scientific basis of preservation by salt and sugar and by drying was firmly established by the results of investigations on the water activities of foods and the way in which they control the growth of microorganisms subjected to a wide range of substrate temperature and composition⁶.

Non-enzymatic browning is often a potent cause of deterioration of stored foods. Major contributions to the elucidation of the complex chemical reactions and the role of the inhibitor, sulphur dioxide, have been made by one of the Division's research teams⁷.

Some of the advances led to developments in the applied field; for example, scientists at the Division used new knowledge from plant physiology when making a device for the rapid determination of the maturity of peas for freezing or canning.

In 1961, large and well equipped new central laboratories were opened at North Ryde, on the outskirts of Sydney, and four years later special funds from the meat industry permitted the building of a new and much enlarged meat research laboratory at Brisbane. In the decade after the Second World War the Division was successful in placing branch laboratories in regions with special problems: a laboratory which was to grow quickly in strength and effectiveness was placed in the New South Wales coastal area to work on remedies for citrus wastage and fruit-fly infestation; another was placed in a fishing port on the southern coast of New South Wales. A strong unit was set up at Hobart, Tasmania, to give technical assistance to the important local fruit and vegetable industries and fisheries; this unit was called in to advise the State Government recently, when levels of heavy metals in Tasmanian fish and oysters were causing worry to the authorities. After the Second World War, in addition to its research activities, the



Central Laboratories, CSIRO Division of Food Research, North Ryde, New South Wales.

Division helped to establish departments of food science at tertiary institutions in Australia, and in the course of several decades it trained many Colombo Plan and other research students from the developing countries.

When Vickery retired in 1967 he could reflect with both satisfaction and irony that it was probably only in the previous 20 years that the Division had achieved the full stature that the Executive had envisaged for it in 1926. Its activities, however, were still somewhat unbalanced, 40% of research effort being devoted to foods of plant origin and only 25% to foods of animal origin, a state of affairs that scarcely did justice to the importance of animal foods in the Australian economy. After M. V. Tracey, former leader of the CSIRO Wheat Research Unit, became the Division's second chief, the balance was rectified by an infusion of money and workers into meat research, and by the amalgamation of the former CSIRO Division of Dairy Research. By 1971 the corresponding figures were 20% for foods of plant origin and 55% for foods of animal origin, with the balance, as before, comprising basic chemistry and physics, and work applicable to both types of food.

Dairy research up to amalgamation

In some important respects, dairy research is the antithesis of food research generally; it deals with one raw material instead of many and in most cases it investigates products that have already been processed or preserved by age-old methods, whereas food scientists in general deal with a range of raw materials which often need new, or greatly altered, forms of preservation. It seems reasonable, therefore, that dairy research should exist as a separate specialist group while food research is covering some of the essential groundwork, but should be incorporated later into the larger research group so as to take advantage of wider horizons and bigger resources.

During its 30 years of independent existence the Division of Dairy Research had an important role in bringing improved or sometimes radically new processes to industry and in achieving new understanding of the chemistry and microbiology of dairy products. As dairy technology was already fairly advanced and successful, the Division was from the beginning able to concern itself with the relatively sophisticated problems of quality (particularly of flavour) and uniformity of dairy products, with methods of reducing the labour cost of manufacturing operations and with the possibility of creating acceptable new foods from the constituents of milk.

In its early years, led by W. J. Wiley, the group investigated problems of butter, which then used 80% of all milk produced in Australia. It was successful in eliminating serious causes of tainting and in showing industry that some traditional steps of butter making could safely be left out, with consequent increase in production.

In 1946 G. Loftus-Hills became Chief of the Division of Dairy Research and remained so throughout the Division's independent life. After it occupied new laboratories at Highett, Victoria, in 1955, the Division began to apply the principles of chemical engineering processes to dairy manufacture. One of the most challenging projects of the group was to introduce mechanisation into the age-old art of cheesemaking. A team succeeded, contrary to most predictions, in mechanising all steps in the process of cheddar cheesemaking and, by this advance and others, wrought a transformation in the Australian cheese industry⁸. Similar success was achieved in the development of a continuous process for the manufacture of casein and subsequently for the recovery of the 'coprecipitate', the total proteins of milk. Efforts to make new foods from milk led to the development of special products for infants with phenylketonuria and with lactose intolerance, and to the manufacture of a high protein milk biscuit that has found acceptance in some underdeveloped countries. The Division of Dairy Research organised the first flavour chemistry group working in Australia, in 1948, and it led the world in identifying the compounds important in the flavour of butter and in isolating compounds

responsible for typical off-flavours⁹. The staff of the division has a particularly successful relationship with industry, ensuring that new developments are fully utilised.

Current interests

From a limited rescue operation for the beef and fruit trades, carried on in a single laboratory attached to the Queensland Abattoir and a few leased cold rooms in the Victorian Government's Cool Stores, CSIRO's interests in food research have developed to embrace nearly all aspects except sugar and margarine. Some recent investigations illustrate the range and quality of current work.

The mechanism of cold injury in plants at temperatures above 0 °C has long been obscure. Work proceeding in the Division of Food Research has shown that the temperature at which a phase change in the cell membranes occurs is indicative of the sensitivity of a plant to chilling injury. Spin labelling techniques have proved to be the best detectors of phase changes¹⁰.

Sophisticated equipment is being used in broadly-based studies of the chemistry of food flavours. Among current achievements have been the identification of the components responsible for the characteristic flavours of peas and passion-fruit¹¹.

Considerable progress has been made in removing limonin, the bitter principle of orange juice, by adsorption on cellulose acetate gel beads and by enzymatic degradation¹².

Valuable additions are being made to knowledge of the sources and control of *Salmonella* infections of meat in abattoirs, and the potential hazards to the health of consumers have thereby been greatly reduced¹³.

Soon after amalgamation with the Division of Dairy Research, the Division of Food Research started the huge project of studying properties of polyunsaturated ruminant foods (made polyunsaturated as a result of a feeding regime developed by the CSIRO Division of Animal Physiology), and of ensuring that the techniques were specified for processing and storing the resulting foods on a commercial scale. The success of this venture, which has made the foods available to people needing to reduce their blood cholesterol levels, would scarcely have been achieved without close collaboration between the Division's three major laboratories.

Future trends

The research programme of the Division of Food Research has hitherto been directed mainly at the solution of the major preservation and processing problems of industry. Greater emphasis is beginning to be given to the needs of consumers and to aspects of human nutrition, and these will be helped by the recent establishment of the CSIRO Division of Human Nutrition.

In this connection, much more work will be done to provide sound microbiological information on which to base realistic legal standards to protect the health of consumers and to assist the processing, storage and handling of perishable foods. Attention will probably also be given to the technical need for various food additives and to their detection in trace amounts in foods. Work on the provision of foods of specified composition to suit people with special dietary needs will be extended beyond the current interest in animal foods with high ratios of polyunsaturated to saturated fats.

Research work aimed at solving waste disposal problems so often encountered by food factories can materially improve the human environment, particularly in rural areas, and may profit the manufacturers: an expansion of this work seems likely.

The urgent needs for mechanisation of the food processing industry are being considered by the Division of Food Research, and an early start is likely on investigations aimed at greatly increased mechanisations of the processes, slaughter, dressing, cooling and cutting up, involved in the conversion of animals into meat.

Increasing emphasis will be given to new sources of proteins for human foods. In addition to studies on the separation and utilisation of blood and whey proteins, these investigations are extending to proteins from seeds such as sweet lupins, which are well adapted to yield profitable crops in a wide range of Australian agricultural conditions.

It is most unlikely that these changes will deflect the Division of Food Research from its continuing purpose in providing essential background information on the physical, chemical and biological properties of foods.

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Minerals research

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CSIRO minerals research has come a long way since its beginning in 1940. I. E. Newnham takes a look at the progress made in that time under the guidance of several strong personalities, and outlines some of the planned future directions.

THE first Executive Committee of the Council for Scientific and Industrial Research (CSIR), later to become the Commonwealth Scientific and Industrial Research Organisation (CSIRO), showed no enthusiasm towards minerals research. Even in its eighth year it spent only £410—of a total budget of £81,000—on the partial support of a mineralogist sponsored by mining companies. Not until its Chief Executive Officer realised the need for a scientific approach to finding new gold-bearing deposits¹ did the council persuade the Treasury to ease the purse strings, and then for four years, CSIR spent £3,000 a year on "gold-mining"; finally, in 1941, it committed itself to a broader programme of minerals research.

It is debateable whether the Council realised the full significance of the step it took when, in 1940, it formed the Division of Industrial Chemistry under a chief (Dr I. W. Wark) who was determined to provide work for the minerals industry. Wark's interpretation of "non-metallic minerals and their utilisation (including ceramics and cement)"—the only Cabinet-approved area of mineral investigation for the new division—was wide and generous. His first staff appointments included Lewis Lewis, a metallurgist, as Divisional Secretary, and R. G. Thomas as Head of a Minerals (later called Minerals Utilisation) Section; together they introduced a comprehensive programme of minerals research. The influence of Wark and Lewis on minerals research policy in CSIR and CSIRO remained unbroken, though not completely unchallenged, from 1940 to 1975.

Early projects and their development

Mineral sands. The first major investigation by the Minerals Utilisation Section concerned the exploitation of the heavy minerals in the beach sands of northern New South Wales. Thomas introduced the subject in his 1941 inaugural staff talk with a characteristic sample of misquoted whimsy:

"The Walrus and the Carpenter were walking hand in hand;

They wept like anything to see such quantities of sand.
'If this were sent to USA', they said, 'it would be grand.'
'If seven maids with seven mops swept it for half a year,
Do you suppose,' the Walrus said, 'that they could get

it clear?'

'I doubt it', said the Carpenter, 'we'd better treat it here!'

"Treating it here" was a major goal of the section.

In an attempt to establish an Australian industry based on the rutile and zircon of Byron Bay, Thomas encouraged research on the chemistry of titanium and zirconium. As a wartime exercise the Division built a small pilot plant for making titanium tetrachloride from rutile and produced titanium metal for high power vacuum tubes. It subsequently initiated a full-scale study of the esters of orthotitanic acid². The discovery that polymerised butyl titanate was an excellent vehicle for heat-resistant pigments resulted in the overseas manufacture and marketing of a new line of paints. No Australian production ensued, however. Nor did it from the development of a process for separating hafnium and zirconium, based on the differential reduction of their chlorides³; although in that case the payment of a substantial patent licence fee by an American company mitigated the disappointment.

Twenty years were to pass before Wark's Division was able to assist the mineral sands industry in the development of the ilmenite upgrading process now used in Australia's first commercial plant. Although not foreseen by Thomas, recent advances in this industrial technology⁴ stem from the work of A. D. Wadsley, who joined the Division in 1943 and attained a world reputation in chemical crystallography before his untimely death in 1969.

Uranium. In 1942 a visit from Sir Henry Tizard, and discussions with Professor Oliphant, alerted Thomas to the need for information concerning Australia's uranium resources. He enthusiastically set out to develop a suitable leaching and recovery process for treating the low grade deposits of Mount Painter in South Australia. Characteristically, his enthusiasm did not extend to the interaction with government departments which the project required; in 1944 he complained to Wark about "the cumbrous and ineffective machinery which characterises interdepartmental liaison in mineral investigation work".

The machinery ran more smoothly when the Premier of South Australia became enthusiastic about the extraction of uranium from the Radium Hill deposit in his state. CSIRO worked closely with the South Australian Government and with the Combined UK-USA Development Agency to perfect a process involving concentration by flotation, acid treatment of the concentrate, and selective ion exchanges of the resulting liquor. An industrial plant, designed and brought into operation mainly by the CSIRO group, was commissioned at Port Pirie in 1954.

Cement. From the outset, Wark sought cooperation between his division and Australian industry. In 1942 he set up a Cement Section under Dr A. R. Alderman to conduct the division's first sponsored research project. The provision of an annual subsidy of £1,500 by the Cement Manufacturers' Association of Australia marked the commencement of a 32-year collaborative study into the manufacture and properties of cements and the preparation of concretes. The major sponsor of the work, which spread into three other divisions, was the Cement and Concrete Association of Australia; it contributed over \$A200,000.

The first success was the recognition of the deleterious effects which occur in cements when active amorphous silica in aggregate particles interacts with the alkali components⁵. The results of this research kept Australia free from major failures such as those which troubled the American cement and concrete industry. Other projects included the use of suitable Australian pozzolanas for the construction of dam walls; the identification of the aggregate rock which had been responsible for cracking and floor slab deformation in the Sydney area; and the formulation of specifications for the correct use of additives to concrete, either to reduce their water content or to increase their fluidity.

Coal. Although Wark established close contact with leading fuel experts from Victoria in 1944, and Lewis promoted the calling of a conference to consider the technical problems of coal use, their initial efforts to launch coal research in the Division of Industrial Chemistry were rebuffed by the Executive Committee. Wark, however, subsequently set up a major project on coal gasification, which culminated in the commissioning of a two-stage pilot reactor for producing high-Btu gas from brown coal⁶.

Meanwhile, the council established a Coal Survey (later Coal Research) Section in Sydney. H. R. Brown, first as Officer-in-Charge and later as Chief of Division, pioneered CSIRO's work on such subjects as the petrography, carbonisation, and inorganic constituents of coal. Studies that elucidated the mechanism by which spheres of 'mesophase' grow during the conversion of vitrinite into coke⁷ attracted international collaboration, and detailed investigation of the inorganic constituents of coal from more than 600 locations in Australia helped to solve problems of boiler-fouling and fly-ash emission.

In 1959 the amount of talent which Wark had assembled reached, so to speak, its critical mass, and one of six fission products, the Division of Mineral Chemistry, merged with the Division of Coal Research in 1967, two years after Wark's retirement. A new member of the enlarged division tracked down the cause of, and provided the remedy for, the inefficient performance of electrostatic precipitators⁸. Coal chemists and petrologists were encouraged to contribute to the techniques of prospecting for oil, gas and minerals in Australia. This group's unorthodox concepts of the genesis of oil and gas from the remains of land plants, in deeply buried sediments subjected to suitable thermal regimes, have proved significant in the understanding of Australian hydrocarbon resources.

Atomic absorption spectrophotometry. A 31-year-old Manchester physicist, Alan Walsh, recruited to the Division of Industrial Chemistry, in 1946, was to become the most spectacularly successful of the many talented scientists who commenced their Australian research careers at 'Fishermen's Bend'. At the time of Walsh's appointment the Chemical Physics Section had acquired a spectrophotometer for molecular absorption spectroscopy, and equipment for atomic emission spectroscopy. Six years later Walsh found himself wondering why molecular spectra were usually obtained by absorption techniques and atomic spectra by emission: his subsequent ideas, and the crucial experiments which followed⁹, started a worldwide innovation. The technique—atomic absorption spectrophotometry—which

Walsh and his coworkers evolved in the following years, has found application in many fields¹⁰. It ranks as CSIRO's major contribution to the minerals industry, and it is noteworthy that Australian mining companies assisted its early development.

Current research

THE establishment of new mineral-processing industries is a laudable, but restricted, objective of the CSIRO minerals research programme. The international availability of know-how, and the widespread technological base of the industry, ensure that in times of normal economic development, individual companies need no encouragement from CSIRO to introduce new mineral-processing industries to Australia. It is true that CSIRO has attempted on several occasions to fill an apparent gap in the range of domestic products by providing novel technology, a recent example being a project for producing electrode carbon by cracking natural gas on the surface of selected brown coal chars. But CSIRO's main role in establishing new areas of production is that of a complementary partner, stimulating and encouraging industry by widespread research. This is not to argue that there is no call for innovative minerals research in CSIRO—indeed, such research is essential in achieving greater technical efficiency for established segments of the minerals industry, and the development of up-to-date investigatory techniques.

CSIRO involvement in these latter areas themselves has been questioned on political grounds, but it has resulted in a close, fruitful relationship with industry¹¹. Current projects include iron ore mining and comminution, the production of iron oxide pellets, the development of International Standards for analytical procedures, the response of flotation plants to changed feed characteristics, the smelting of low grade tin concentrates, and the disposal of coal washery rejects and metal refining by crystallisation and reflux.

Modern instrumental technology is being used to solve problems in areas such as borehole logging, the remote sensing of minerals, and on-line analysis. Techniques that are subject to detailed study include energy dispersive X-ray fluorescence analysis, transient electromagnetics, quantitative X-ray diffraction analysis, fluid inclusions, microanalysis and isotope geochemistry.

CSIRO is also concerned with the creation (in cooperation with State and Federal Government Departments and Agencies) of a technical base which evaluates resources and anticipates the needs of the nation. Though excellent in concept, this aim can be difficult to achieve—consider the misunderstandings which attended recent attempts to promote liaison between CSIRO and the former Department of Minerals and Energy. Fortunately, there has emerged a Joint Department—CSIRO Research Advisory Committee—which will be concerned with the formulation and modification of future mineral research programmes. In addition to its long-standing associations with state mining departments, electricity authorities and geological surveys, CSIRO is currently forging close links between its mineral divisions and various environmental groups at both State and Federal level.

During the past decade the Executive has encouraged divisions to adopt a management system which helps to define the intent and output of CSIRO's research programmes¹². This has resulted in a method of annual reporting which quantitatively identifies the movement of research staff into new areas of investigation. Figure 1 shows the changes that have occurred in the Minerals Research Laboratories during the past five years, as well as those projected for 1976–77. Of the 194 members of the professional staff employed in 1971, 59 have moved into new programmes falling under the categories of 'environment' and 'energy' (Fig. 1). This movement has not occasioned any major disruption of the structure of individual divisions.

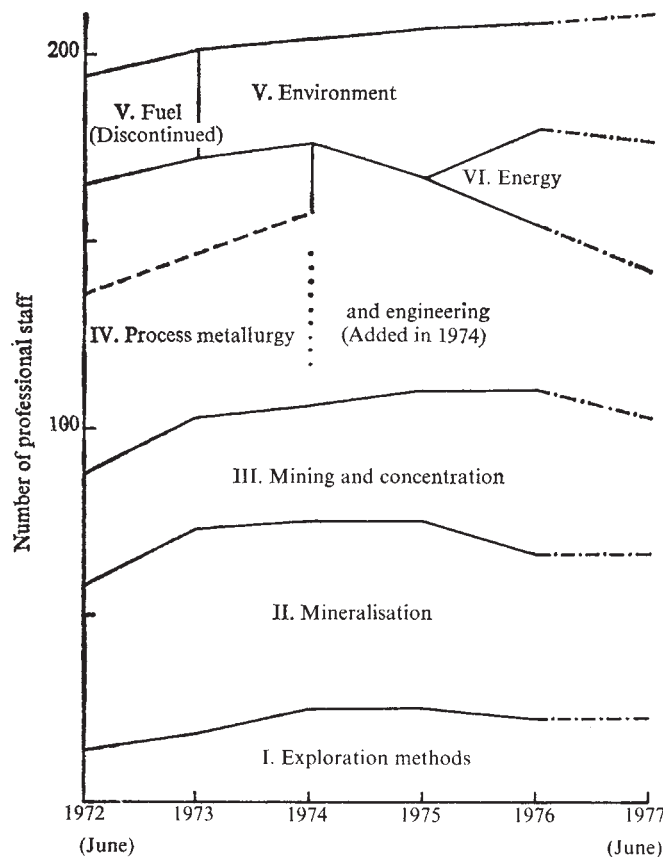


Fig. 1 Professional manpower resources allotted to the programmes of CSIRO Minerals Research Laboratories, 1971–76.

Inorganic chemists do not become geophysicists, or vice versa, but both agree to apply their combined skills to the solution of problems in areas with which they had no previous academic association. Many of these changes originate at the level of the research scientist.

If there is a budding Walsh among the new generation of CSIRO scientists, his Chief can use this flexibility to allow his work to expand in unexpected directions without having to justify such action by extravagant prediction or premature speculation. It is a Chief's responsibility to foster exceptional talent in ways that his own judgment best dictates.

Future trends

A major goal in the next decade will be to cultivate the greatest possible skill in those areas of science that satisfy or anticipate industrial and community needs. No matter how practical these needs may be, efforts to meet them at times lead research workers into realms of academic theory; such movements are deemed essential to CSIRO minerals research.

Several activities in the six major programmes of the Minerals Research Laboratories are, during the next decade, likely to require additional effort by the constituent divisions of Chemical Engineering, Mineral Chemistry, Mineral Physics, Mineralogy and Process Technology.

Exploration methods. There will be a continuing emphasis on improving the technology of exploration. A thick and often saline overburden renders exploration difficult in many parts of Australia. Basic research into electrical geophysics, rock magnetism, the interpretation of aerial photography, and several forms of geochemistry will be linked with the specialised field knowledge and experience of the exploration industry, state geological surveys and the Bureau of Mineral Resources.

Mineralisation. A clear understanding of the genetic environment of an economic mineral deposit, if it can be

reliably associated with chemical and/or physical features of the rocks, may form the basis of a valuable exploration technique. Because Australian geology shows many similarities to that in parts of India and southern Africa, there is a possibility of finding Australian equivalents to the economic deposits of these other countries. A prime target is the discovery in the north-west of the continent of gold-uranium deposits analogous to the 'banket' of the Witwatersrand. The art of prospecting still awaits the solid reinforcement of academic theories on Precambrian environments; CSIRO can help to build these theories and develop them into practical advice for the exploration industry.

Mining and concentration. The development of new mining methods—particularly the application of computer techniques to the solution of underground problems—must be studied in Australia. The extraction of coal from thick seams at depth requires urgent investigation. There will be an increasing emphasis on process control, particularly as new and better on-line sensors are developed, and computers become smaller and cheaper. A system will be developed for extracting on-line positional and compositional information from particles whose image is created by the beam of a scanning electron microscope.

Process metallurgy and engineering. This programme is concerned with the transformation of minerals into materials of increased value and of greater marketability. In the important field of pellet formation there will be some need for research on the roles of solid state diffusion, slag formation and migration, and bonding between the liquid and solid phases. The gradual depletion of existing reserves will necessitate the development of hydrometallurgical and pyrometallurgical methods for treating low grade complex ores. There will be an increased need to study high intensity pyrometallurgical reactors.

Environment. Increased research effort will be devoted to understanding the influence of industrial and chemical processes on the biosphere and, where possible, to ameliorating any undesirable effects. In particular there is a need for field studies supported by laboratory work on photochemical smog, urban haze, pollution by sulphur dioxide and nitrogen oxide, and the effect of heavy metals on aquatic life.

Energy. There will be continued emphasis on longer term projects related to oil and gas exploration, the efficient mining of coal, the possible conversion of coals into liquid fuels, alternative energy sources and energy storage. Insufficient knowledge of the nature and location of source materials, and of the conditions controlling hydrocarbon generation and accumulation, is available to guide exploration in off-shore areas of the North-west Shelf and Bass Strait. CSIRO will continue to provide data and ideas to support the companies exploring these fields and certain inland basins. The complicated and variable constitution of coal makes it essential that fundamental work should accompany practical studies of the most suitable process, or combination of processes, for converting coal into oil in Australia. There is an urgent need to understand the chemical and physical properties of those fractions of Australian coal which are enriched in particular macerals, and to assess the influence of heating rate on both the initial and subsequent processes of depolymerisation which affect the yields of tar and gas.

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Wool structure and biosynthesis

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Originally set up to assist the textile processing and manufacturing industries, the CSIRO Wool Research Laboratories have contributed significantly to fundamental knowledge of the chemistry and biology of wool.

"Captain Macarthur considers it his duty respectfully to represent to His Majesty's Ministers, that he has found, from the experience of many years, the climate of New South Wales is peculiarly adapted to the increase of fine-woolled sheep . . . Captain Macarthur being persuaded that the propagation of these animals would be of the utmost consequence to this country, procured in 1797 three rams and five ewes, and he has since had the satisfaction to see them rapidly increase, their fleeces augment in weight, and the wool very visibly augment in quality."

Thus wrote Captain John Macarthur to Lord Hobart in 1803¹. His prophecy was amply fulfilled—the sheep population of Australia currently exceeds 150 million and the annual production of wool is around 770×10^6 kg with a value of £620 million. Research for the benefit of the wool industry is financed through grants from a Wool Research Trust Fund which is replenished partly by a levy on the sale of wool and partly by direct contributions from the Australian Government. The fund provides grants for both promotion and research and the bulk of the research is carried out by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). The grant to CSIRO for wool research during the financial year 1975–76 was approximately £8 million.

During the first two decades after the formation of the Council for Scientific and Industrial Research (CSIR), wool research was concerned primarily with production and the main benefits to the grower were in terms of increased production and improved quality. Towards the conclusion of the Second World War, production of both synthetic and regenerated man-made fibres increased and this was seen as a threat to wool's future as a textile fibre. Accordingly, CSIR in 1945 invited four leading textile scientists to advise on the establishment of a facility in Australia for wool textile research, with the aim of assisting the wool processing and manufacturing industries both in Australia and overseas. One of the advisers was J. B. Speakman who, at that time, was Professor of Textile Technology in the University of Leeds. He recommended the establishment of a protein research laboratory devoted to the study of the chemical and physical structure of wool and its chemical reactivity, a textile engineering research laboratory, a textile technological research laboratory and a wool grease research laboratory. In 1948 the decision to undertake wool textile research in Australia was implemented with the establishment of three laboratories which today constitute the CSIRO Wool Research Laboratories; the Division of Protein Chemistry situated at Melbourne, the Division of Textile Physics situated at Ryde in New South Wales, and the Division of Textile Industry situated at Geelong in Victoria, which handles studies of wool grease as part of its programme. Australia was not alone in seeing the need for wool-growing countries to be concerned with the end use of the product as well as its growth, and since 1964 wool textile research in South Africa, New Zealand and Australia has been coordinated through the Research and Development Committee of the Board of the International Wool Secretariat.

Wool has a number of characteristics which are highly desirable in a textile fibre. It can readily be dyed to a wide range

of colours and shades, it can be set into desired shapes and yet shows good wrinkle resistance and recovery, and it can be compacted when required by controlled felting. It is also a very safe fibre compared with cotton and synthetics since it tends to char rather than burn and when ignited burns only slowly. Wool garments have outstanding heat insulation properties and yet allow free movement of moisture and air, thus making them comfortable to wear. On the other hand, untreated wool is susceptible to attack by larvae of the clothes moth and is damaged by prolonged exposure to sunlight, and untreated wool garments shrink and lose their set when washed or exposed to warm, humid conditions. The aim of studying wool structure and biology has been, first, to provide background knowledge to serve as a basis for understanding wool's properties, both good and bad, and second, to suggest ways in which these properties might be modified to advantage. At first sight it might be thought that studies of the physical and chemical nature of wool alone might be sufficient. But wool is a highly variable product and the rate of wool production, the distribution of fibre diameters, the crimp or curl, the mechanical properties and colour are all affected by the health, nutritional state and environment of the sheep and so in turn are the processing characteristics of the wool. Studies of the biology of wool growth thus form an essential adjunct to structural studies in providing background knowledge for the benefit of the wool textile technologist, quite apart from their intrinsic value in wool production.

The foundations of present knowledge of the molecular structure, mechanical properties and chemical reactivity of wool were laid in the early 1930s by W. T. Astbury and H. J. Woods and by J. B. Speakman, all of whom worked at the University of Leeds. Wool is naturally insoluble due to the presence of covalent disulphide linkages between the molecular chains, but by 1935 D. R. Goddard and L. Michaelis working at the Rockefeller Foundation in New York had isolated relatively intact proteins from wool solubilised by reduction. The remarkable complexity of the wool fibre first became apparent after the low-angle, X-ray diffraction studies of I. MacArthur at Leeds, and the early electron microscope studies at the CSIRO Division of Chemical Physics in Melbourne. The formation of the CSIRO Wool Research Laboratories provided, for the first time, facilities for an integrated programme of research into the mechanical properties, molecular structure and chemical reactivity of wool. In addition, it provided an opportunity for research results to be exploited for the benefit of the wool industry within the one group of laboratories.

It is not possible here to review adequately the extensive programme of research on wool biology and wool textiles that have been carried out in the CSIRO laboratories. As indicated by the title, this article is concerned primarily with wool structure and biosynthesis and the following sections give a brief account of the current state of knowledge on these topics.

Biosynthesis

In 1927, shortly after the formation of CSIR, a laboratory was established in Adelaide for the study of the influence of nutrition on the biochemistry and physiology of the sheep. The basic knowledge acquired in this study revealed that animal health and wool production in a number of areas were adversely affected by deficiencies of copper, cobalt and zinc in the soils. Spectacular improvements resulted when traces of the deficient

metals were supplied either as a dietary supplement or in a superphosphate top-dressing to the soil. At present research which bears either directly or indirectly on wool biosynthesis is being carried out in many CSIRO divisions. Investigations of soil, pastures, diseases and parasites, genetics, flock husbandry and management, and physiology of sheep reproduction, nutrition and wool growth have all contributed to more efficient wool production.

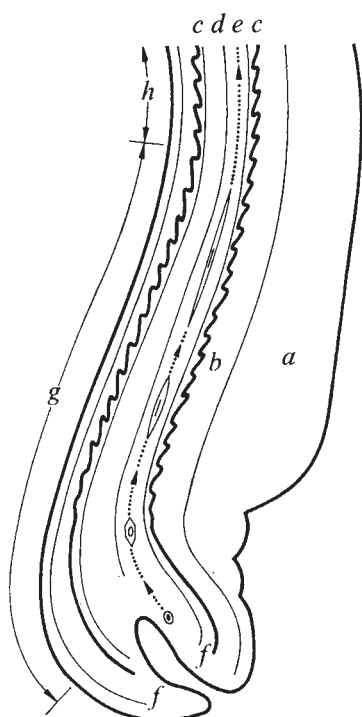
In most mammals the pelage consists of an outer layer of coarse, heavily medullated fibres and an inner layer of much finer fibres. In the case of fine-woolled sheep the coarse outer layer is absent. Another difference is that most mammals have a growth-replacement cycle but in fine-woolled sheep the growth is effectively continuous.

Wool is produced in follicles formed by invagination of the epidermis and at the base, or bulb, of the follicle there is an invagination of the surrounding connective tissue termed the papilla (Fig. 1). In the region surrounding the papilla proliferation of cells destined to form the fibre and the inner root sheath occur. The central stream of cells elongate during their passage through the follicle and form the cortex of the wool fibre. A second stream of cells, surrounding the first, become flattened and form the cuticle of the fibre. No cell divisions are observed in either of these streams above the bulb region. Additional cell streams destined to become the inner and outer root sheaths envelop the fibre cells.

Wool proteins, termed keratins, are synthesised and laid down intracellularly as the fibre cells progress up the follicle. The cortical cells fill with fine filaments which are embedded in a cystine-rich matrix. The available evidence suggests that the synthesis of filament and matrix proteins takes place concurrently but the relative rates of production do not seem to be equal, with the relative rate of production of the matrix proteins increasing sharply during the latter stages of biosynthesis.

Initially the keratin proteins are laid down in the thiol form but as the fibre cells mature the cysteinyl residues are oxidised in pairs in a copper-catalysed reaction and the resulting disulphide cross linkages render the structure insoluble in the natural environment and resistant to attack by microorganisms.

Fig. 1 Diagrammatic longitudinal section (foreshortened) of a fine-wool follicle. *a*, Outer root sheath; *b*, inner root sheath; *c*, fibre cuticle; *d*, paracortex; *e*, orthocortex; *f*, undifferentiated region; *g*, zone of maturation of fibre cells; *h*, fully formed fibre.



This process is termed keratinisation.

During the passage between the bulb region and the zone of keratinisation a layer of material is deposited between the cells and the cell membranes are modified. In the final fibre the resulting complex effectively binds the keratin in the individual cells into a mechanically continuous material. The combined effects of disulphide cross linking and deposition of the cell membrane complex give wool a high temperature stability and the effect of temperature on the initial stretching modulus of wool in water is only slight up to 100 °C.

Breed and nutrition have an important influence on the processing and textile properties of wool and research has been undertaken in the Division of Textile Industry to compare the processing properties of a range of wools and also the fabrics produced from them. Fibre diameter was found to be the most important single factor affecting the so-called handle or softness of the fabric, with the finest wool giving the softest fabric. For wools of a given diameter range the fabric properties were generally insensitive to variations between wools but the processing properties varied considerably. In addition to direct practical tests, basic studies of the biosynthesis of wool have been carried out in the Division of Animal Physiology and in the Division of Protein Chemistry with the aim of understanding the effects of breed and nutrition on fibre production, structure and properties.

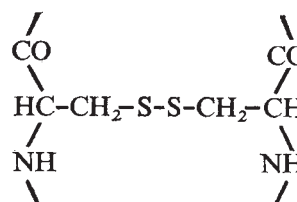
Rate of wool growth depends on the genetic background of the animal, its nutritional status and a number of physiological factors. Experiments carried out at the Division of Animal Physiology have established that both wool production and the sulphur content of the wool are influenced by the availability of sulphur-containing amino acids in the diet. This was controlled by intra-abomasal feeding and the content of high sulphur proteins in the wool varied within the range 18–34%. A number of dietary treatments, for example feeding with cereal proteins, have been found to suppress production of certain glycine-rich matrix proteins but so far none has been found to increase their proportion.

In animals maintained on a copper-deficient diet the copper-catalysed cross-linking stage in keratin synthesis is defective and a grossly abnormal fibre is produced. A similar situation, associated with genetic defects, occurs in both mice and men.

The product of the wool follicle is very unusual in that both the proportions of the individual proteins and the cross linkages between them can be manipulated within wide limits by dietary means. Potentially this provides a method of varying the properties of wool for particular end uses. A wide variety of agents are known which will suppress wool growth. Most are toxic, but in recent years studies have been carried out in the Division of Animal Production to test the feasibility of "chemical shearing" in which the animal is either injected with or fed a substance which causes a temporary interruption of wool growth and subsequent shedding of the fleece.

Wool proteins

Wool is almost entirely composed of cystine-containing proteins termed keratins and, as mentioned earlier, the natural insolubility of wool is attributed to the presence of interchain disulphide linkages formed by post-synthetic oxidation of pairs of cysteinyl residues (I).



A major programme of the Division of Protein Chemistry has been concerned with the solubilisation of wool and the prepara-

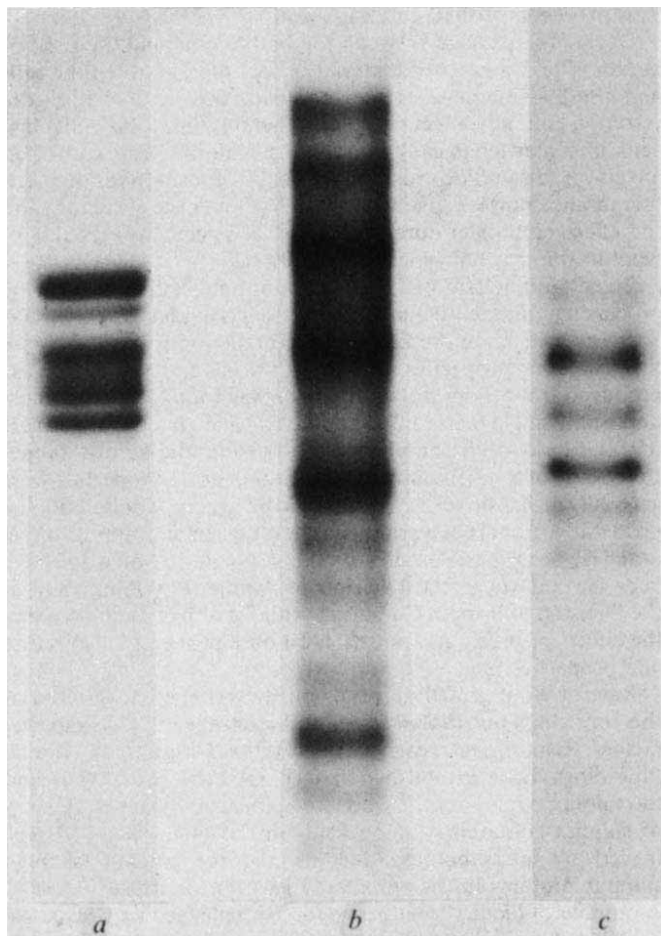


Fig. 2 Electrophoretic patterns of the three groups of wool proteins. *a*, Low sulphur group run in sodium dodecyl sulphate-polyacrylamide gel, pH 7.0. *b*, High sulphur group run in polyacrylamide gel in urea buffer, pH 2.6. *c*, Glycine-rich group run on cellulose acetate in a urea buffer, pH 8.9. (Division of Protein Chemistry.)

tion of derivatives suitable for the analysis of protein composition, and the purification and characterisation of individual protein components. Care must be taken to prevent peptide bond hydrolysis during the procedures used for breaking disulphide bonds and the subsequent extraction of proteins. Much early work was concerned with the evaluation of different methods for breaking disulphide bonds, and procedures were developed using peracetic and performic acids, tributyl phosphine, oxidative sulfitolysis, sodium thioglycollate and mercaptoethanol. In more recent work the latter two reagents have generally been used and the extracted protein subsequently treated with sodium iodoacetate to give the *S*-carboxymethyl derivatives. This prevents reformation of the disulphide linkages and also improves solubility.

Three main groups of wool proteins have been identified and isolated. Proteins in the low sulphur group (Fig. 2*a*) have fewer cysteinyl residues per cent than whole wool and have molecular weights in the range 40,000–58,000. In solution the *S*-carboxymethyl derivatives of these proteins have an α -helix content of about 50% and helix-rich chain segments about 100 residues long can be isolated after partial hydrolysis with proteolytic enzymes. Two such segments have been sequenced and the distribution of non-polar residues was found to have the 7-residue periodicity predicted by F. H. C. Crick for the coiled-coil conformation. Oriented films of the fragments exhibit the characteristic X-ray diffraction pattern of the coiled-coil rope structure.

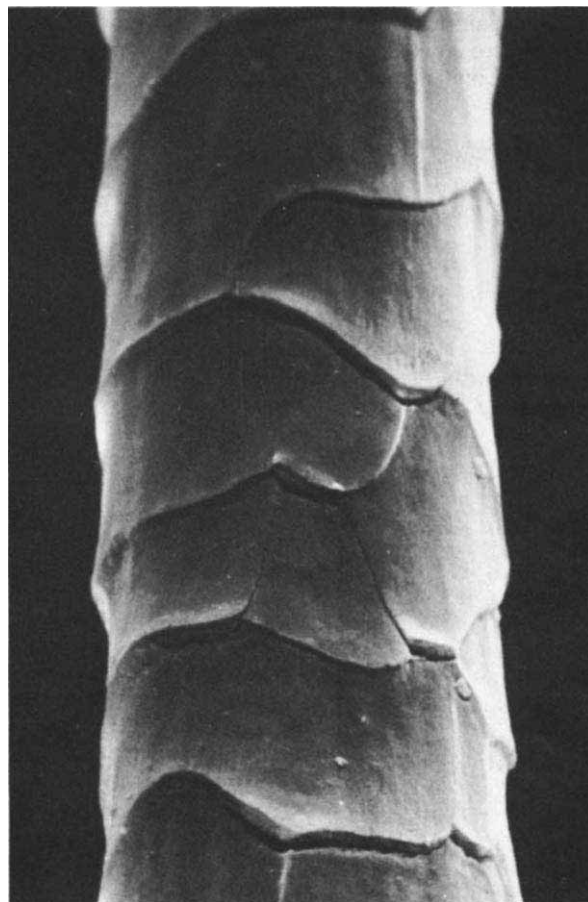
Proteins in the high sulphur group (Fig. 2*b*) have more cysteinyl residues per cent than whole wool and have molecular weights principally in the range 10,000–28,000. In solution

the *S*-carboxymethyl derivatives of these proteins do not appear to contain any α helix. The total number of high sulphur proteins in a wool fibre probably exceeds 100 and various subgroups have been recognised. Proteins from the low and intermediate molecular weight subgroups have been sequenced by South African wool scientists while several proteins in the upper molecular weight range have been sequenced in the Division of Protein Chemistry. These latter sequences exhibit a slightly imperfect decapeptide repeat, the structural significance of which is unknown.

Proteins in the third group (Fig. 2*c*) are characterised by a high content of glycyl residues (up to 40%) and a higher than average content of aromatic residues (up to 34%). The number of glycine-rich proteins in a wool fibre probably exceeds 50 and two subgroups have been recognised which differ in their contents of cysteinyl residues and phenylalanyl residues. The molecular weights of the glycine-rich proteins are generally below 10,000 and in solution the *S*-carboxymethyl derivatives do not seem to contain any α helix. So far only one glycine-rich protein has been sequenced and the only evidence for regularity is in the occurrence of segments in which glycyl residues alternate with non-glycyl residues. A similar alternation is found in silk fibroin from the silk moth *Bombyx mori* and is there associated with a special mode of packing of β -pleated sheets. The significance of this alternation in the case of wool proteins is unknown.

Although much progress has been made in the isolation and characterisation of wool proteins there is virtually no evidence concerning specific interactions between proteins in different groups. In such a highly organised structure as wool it is likely

Fig. 3 Scanning electron micrograph of the surface of a fine wool fibre showing the projecting edges of the cuticle cells. The direction of the tip is towards the bottom of the micrograph. (Division of Textile Industry.)



10 μ m

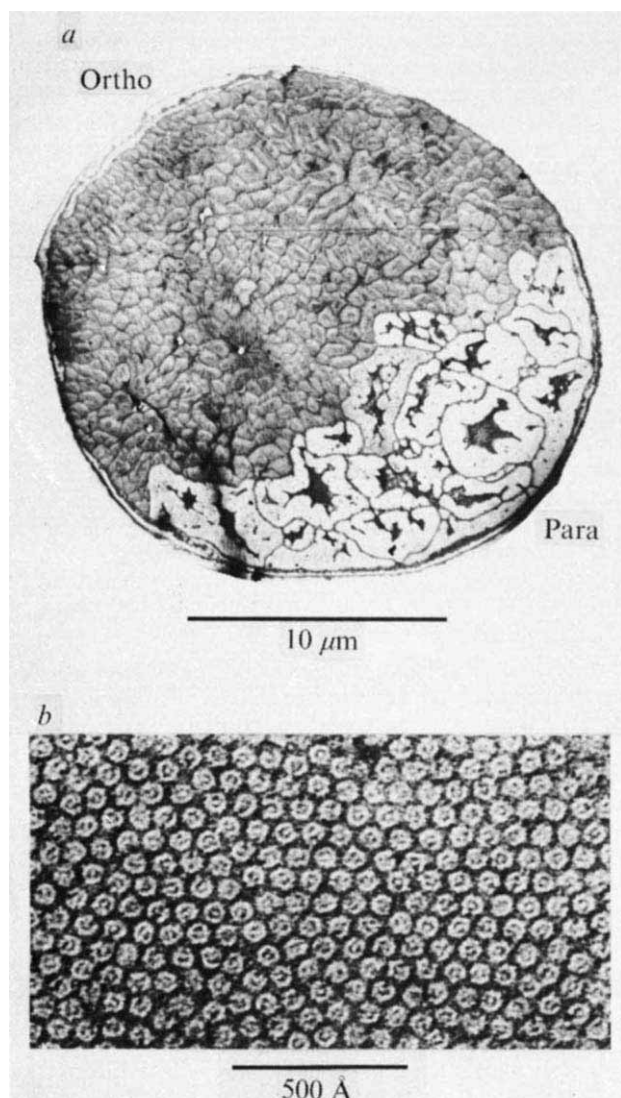


Fig. 4 Electron micrographs of cross sections of fine wool. *a*, Low power micrograph showing the cuticle, and the orthocortex and paracortex. *b*, High power micrograph showing the filament-matrix texture. (Division of Protein Chemistry.)

that aggregates of precise stoichiometry are present and one aim of present research is to determine their nature.

Wool structure

A wool fibre from a Merino sheep is typically 20–30 μm in diameter and the exterior portion, amounting to about 10% of the weight, consists of a layer of flattened cells which overlap to form a continuous tube termed the cuticle (Fig. 3). The projecting edges of the flattened cells are believed to play a part in anchoring the fibre in the follicle and in excluding foreign matter from the fleece. In addition they give wool its unique property of felting. This property is valuable in that the density of a fabric can be increased by mechanical means but the same property leads to the familiar felting shrinkage when untreated woollen garments are washed. Several new processes for preventing felting shrinkage have been developed in the Division of Textile Industry and improvements to the dry chlorination process have been made in the Division of Protein Chemistry. All involve masking or modifying the projecting cell edges and in some instances fibres are also chemically "spot welded" to prevent the irreversible relative movement between fibres which leads to shrinkage. The membranes around the cuticle cells are consolidated during biosynthesis to form an outer envelope around the fibre which is termed the epicuticle. Although very thin, this layer appears to influence

the textile properties since dyeing proceeds more rapidly when the epicuticle has been damaged mechanically or chemically. The chemical composition of the cuticle differs from that of the remainder of the fibre, and cuticle cells do not seem to contain any low sulphur proteins. The bulk of the cuticle is composed of high sulphur proteins and work is in progress to compare the high sulphur proteins of the cuticle with those present in the rest of the fibre.

In coarse animal hairs the main body of the fibre, enclosed by the cuticle, consists of a dense annular cortex and a central vacuolated medulla. In fine wool the medulla is absent and so the cortex occupies the entire volume enclosed by the cuticle. The cortical cells are highly elongated and in fine, highly crimped wools, which are the main product of the Australian sheep industry, the cells are of two distinct types and are grouped together as shown in Fig. 4*a* to form a segmented filament. The topographical relationship between the segments is such that one, termed the orthocortex, is always on the outside of the crimp wave while the other, termed the paracortex, always remains on the inside of the crimp wave. The chemical and physical properties of the orthocortex and the paracortex are quite different and wool scientists in numerous laboratories throughout the world have sought to devise methods of isolating and characterising the two types of cortical cell. No definitive answers have yet been obtained but there is some evidence that the differences in properties are due in part to differences in protein composition, with the paracortex containing more high sulphur protein than average and the orthocortex containing more glycine-rich protein than average.

Electron microscope studies reveal that the cortical cells have a filament-matrix texture (Fig. 4*b*) and characteristic differences between the filament packing in the orthocortex and in the paracortex have been noted by both Australian and other wool scientists. Although much has been written about the orthocortex-paracortex structure it must be remarked that a clear understanding of its relation to crimp has not so far been obtained. In view of the importance attached to crimp by wool buyers this is clearly a field in which a sustained research programme is warranted.

Much circumstantial evidence has accumulated that the filaments in the cortex, termed microfibrils, are composed of low sulphur proteins and that the matrix is composed of high sulphur proteins. Recently it has proved possible to isolate microfibrils in a purified state, and to demonstrate directly that they contain low sulphur proteins. The glycine-rich proteins are only a minor component of most wools and their location was until recently unknown. After the discovery that some epidermal appendages such as quills contain high proportions of glycine-rich proteins it was possible to show by means of a combined quantitative extraction and low-angle X-ray diffraction study that the bulk of the glycine-rich proteins reside in the matrix.

Much use has been made in both the Division of Protein Chemistry and the Division of Textile Physics of X-ray diffraction in the study of the structure and mechanical properties of wool. The wide-angle diffraction pattern of wool has been extensively studied in the Division of Protein Chemistry and shown to be well accounted for by supposing that the α -helices in the low sulphur proteins are twisted together to form coiled-coil ropes. The low-angle equatorial diffraction pattern is determined by the axial projection of the microfibril and also by the packing arrangement of the microfibrils, and an understanding of this pattern has enabled estimates to be made of microfibril diameter and also the mean distance between nearest neighbours. Comparison of the patterns for dried and hydrated wool revealed that most of the water taken up by the wool fibre is accommodated in the matrix. This suggests chemical modification of the matrix as a likely method of influencing the many properties of wool that are humidity dependent.

Great interest attaches to the determination of the way in which the low sulphur proteins are assembled to form the microfibril. This information is contained in the X-ray diffraction pattern of wool but interpretation is hampered both by the very large axial identity period of the microfibril and by the lack of information on the stoichiometry of the low sulphur protein molecules. The most recent studies suggest that the microfibril has a helical symmetry with a repeating group of

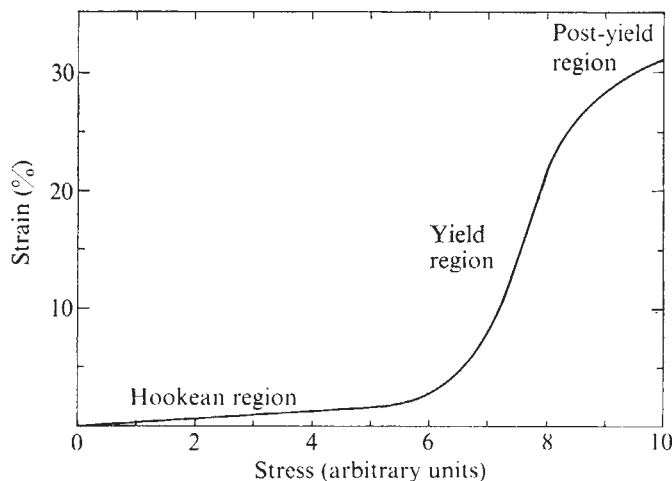


Fig. 5 Stress-strain curve for the extension of a single hair in water. (Division of Textile Physics.)

low sulphur proteins disposed at axial intervals of 67 Å on a helix of pitch 220 Å. The molecular weight of the repeating unit is estimated to be about 230,000, corresponding to four to six molecules. As with many helical structures there is evidence of periodic axial distortion.

Mechanical properties

Composite filament-matrix textures are common in nature and used widely in man-made structures. In general the filaments are composed of a material with a high modulus of elasticity and the matrix of a material with a low modulus of elasticity. The special advantage of such a texture is the behaviour under stress. The matrix, which is easily deformed, distributes the stress evenly over the filaments thus preventing the propagation of cracks from local imperfections or points of rupture. Studies carried out in the Division of Textile Physics highlight the added subtlety in the case of wool that the elastic modulus of the matrix is highly dependent on water content. In a dry fibre the modulus approaches that of the filaments, but in a fully hydrated fibre the matrix behaves like a highly swollen gel. This observation provides the key to understanding many important features of the processing properties and performance of wool as a textile fibre.

A typical stress-strain curve for a naturally straight keratin fibre (hair) is shown in Fig. 5. In the case of wool there is an initial region of high slope in the stress-strain curve associated with decrimping of the fibre. Up to 2% strain, the relationship between stress and strain is linear (Fig. 5) and the strain is accommodated in the individual protein molecules. Beyond 2% strain the fibre yields readily with increasing stress but stiffens up again at about 25% strain. X-ray studies suggest that the microfibrils can only accommodate about a 2% strain before some breakdown occurs in the helical structure. Above about 2% strain there is a progressive change in the high-angle X-ray diffraction pattern with the appearance of new reflections associated with the β conformation. The transformation from the α conformation to the β conformation has been studied in

some detail in the Wool Research Laboratories because of its relevance to setting. Set is defined as the strain retained after the removal of an applied stress and may be temporary or permanent. Temporary set is generally undesirable since it leads, for example, to wrinkling; permanent set is a useful property which can be used to shape clothing.

Extensive studies of the viscoelastic properties of wool and their dependence on temperature and hydration have been carried out in the Division of Textile Physics to gain an understanding of the associated textile properties. Methods of measuring fabric properties have been developed at the Division of Textile Industry and used in the development of methods for producing durable pleats and creases in woollen fabrics and for conferring wash-and-wear properties. The influence of chemical reagents on the viscoelastic properties of wool have also been extensively studied in the Wool Research Laboratories. In particular the facilitation of conformational change by a thiol-disulphide interchange reaction, first noted by R. W. Burley, has been found to be an important factor in creep and stress relaxation.

Chemical reactivity and future research

In his report to CSIR in 1945, Speakman recommended that "... first place in any scheme of research intended to promote the utilisation of wool must be given to organo-chemical studies of the reactivity of the keratin molecule" and extensive programmes of research have been carried out both in the Division of Textile Industry and in the Division of Protein Chemistry on reactivity of wool in relation to bleaching, dyeing, sunlight yellowing, removal of vegetable matter by carbonising, enzymatic hydrolysis, interactions with polymers, resistance to insect damage and flammability. In some cases the approach has been semi-empirical, in others extensive use has been made of the basic knowledge gathered on the structure of wool.

The aims of research on wool structure have been to accumulate basic knowledge concerning the molecular structure, mechanical properties and chemical reactivity of the wool fibre and to relate this to the processing and textile properties of wool. The aims of research on the biosynthesis of wool have been to obtain an understanding of the physiological and nutritional factors that influence wool growth and quality.

Much has been achieved during the past 50 years. Some research results have found immediate practical application, some have provided basic knowledge of wide applicability and some have merely served to draw attention to our ignorance. Some of the major gaps that need to be filled by future research concern the basis of the regulatory control of the biosynthesis of the keratin proteins, the arrangements of the protein molecules in the microfibrils and in the matrix, the distribution of disulphide linkages within and between the protein molecules, and the influence of structural variations on textile properties. In addition there is an urgent need for more information about the structural basis of the photochemical degradation of wool, wrinkling, setting and about the relationship between fibre and fabric properties.

When these objectives have been attained the way will be open to modify wool's properties in a predictable manner either by control of synthesis or by chemical or physical treatments.

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Fifty years of plant research

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Over the past 50 years the present Division of Plant Industry has evolved from relatively modest beginnings as the Division of Economic Botany with a brief to undertake basic research in plant sciences, to a Division whose work now touches on many aspects of crop and pasture plants, as well as the natural vegetation of Australia.

At the time of establishment 50 years ago of the Council for Scientific and Industrial Research (CSIR), the agricultural and pastoral industries were predominant in Australia's economy. The most pressing research problems were in the field of agriculture, and these were the concern of all the first laboratories to be established. The Departments of Agriculture of the six States had been actively engaged in agricultural research for many years. Keenly aware of the need to secure their co-operation, Rivett arranged a meeting with the Directors of Agriculture, which agreed that CSIR should concentrate on problems "which are regional or national in scope, fundamental in character, and which require concentration of effort and highly specialised research for their solution". The introduction and nutrition of plants, genetics and the biology of plant diseases were cited as investigations suitable for CSIR, whereas fields such as plant breeding and the control of plant diseases were considered more appropriate for the State Departments. It was within this somewhat arbitrary framework that the Division of Economic Botany, now called Plant Industry, began its work.

Plant diseases

B. T. Dickson, a plant pathologist who came from Canada to be the first Chief of the Division (1928–50), initiated research on a wide range of plant diseases, including take-all and flag smut of wheat, blue mould of tobacco, and water blister of pineapple. Virus-free stocks of potatoes were released, with a substantial impact on yield, and the possibilities for genetic or chemical control of several diseases were explored.

The most striking early success was in the control of blue mould of tobacco, caused by the fungus *Peronospora tabacina*, which threatened to wipe out tobacco growing in Australia. H. R. Angell found that exposure of seedlings to benzol vapour provided effective control, and this practice undoubtedly saved the infant industry following its introduction in 1935. Recent research on blue mould has concentrated on the breeding of resistant varieties. Sources of resistance to the two predominant strains of the organism were located in some of the wild Australian species of *Nicotiana*, and have been transferred to cultivated tobacco, so that resistant cultivars now account for nearly all of the tobacco currently grown in Australia.

Another disease, of current concern throughout Australia, is the dying back of many species of eucalypts and other trees caused by the fungus *Phytophthora cinnamomi* and related organisms. As with blue mould, there has been a differentiation of strains by the pathogen and of resistance by the host, with important implications for their ecology and control. Such soil-borne diseases, and the complex microbial interactions involved in them, will probably require more of our attention in the future.

Apart from research on specific diseases, the Canberra plant pathologists have been active in developing the concept of

phytoalexins, compounds which are produced by the host plant in response to infection and which then limit the development of the pathogen. This concept was first put forward by K. O. Müller, a Research Fellow in the Division for many years, and his colleagues subsequently isolated and identified pisatin and other phytoalexins. A polypeptide which elicits phytoalexin formation has also been identified and much is now known of the biology of the response system, but to what extent these defensive reactions can be used to control plant diseases remains to be seen.

Introduction of useful plants

The native species of *Nicotiana* have already proved of value in breeding programmes, and there is much international interest in Australian species related to cotton, soybean, sorghum and rice as sources of useful genes. The aborigines did not domesticate any plants, however, and all the crops and improved pasture plants used in Australia have come from overseas, several having been brought into the country by chance in earlier years.

Deliberate plant introduction has been a major activity of the Division since its establishment, our accessions numbering more than 70,000 out of an Australian total of about 100,000. Cereals and temperate zone pasture plants predominated in the early years, then grasses and legumes of potential value in the more tropical pastures of northern Australia, while currently the major emphasis is on crop plants. The Division has mounted 12 collecting expeditions since 1947, the first two being to South America and the Mediterranean, the most recent to the USSR.

The many introduced pasture plants were evaluated in the Division's network of field stations throughout Australia, and cooperatively with State Departments of Agriculture. More than 70 introductions have proved to be of direct commercial value as pasture plants, and many others have been used in plant breeding programmes. For example, legumes better adapted than subterranean clover to the drier and more alkaline soils of the wheat belt are now available as a result of the introduction and hybridisation of a range of medics from the Mediterranean region.

Land surveys and pasture research

Pasture improvement has always been a major concern of the Division. The native grasses were studied initially, and a survey of Australian grasslands was undertaken. One result was a sharper realisation of how little was known of the agricultural potential of northern Australia, and several field stations were established there. A series of land-use surveys was made under C. S. Christian, whose unit was eventually established as a separate Division of Land Research.

Agrostological research also grew rapidly in the 1940s under J. Griffiths Davies. New regional laboratories and field stations were set up throughout Australia. In the south, the finding of better ways to establish subterranean clover and of more effective fertiliser practices were crucial to the rapid extension of improved pastures. In the north, where the group under Davies transferred and eventually became the Division of Tropical Pastures, the introduction and improvement of a wide range of exotic grasses and legumes was a key element in their success.

More recent pastoral research at Canberra includes the breeding of improved legumes and grasses, such as cultivars of *Phalaris tuberosa* with greater seed retention and seedling vigour



The first three chiefs of the Division of Plant Industry: from the left, Sir Otto Frankel (1951–62), Dr B. T. Dickson (1928–50) and Dr J. E. Falk (1963–70).

and reduced tryptamine content. The improved plants have been assessed by measurements not only of pasture growth but also of the productivity of animals grazing on them. Computer simulation techniques are currently being used to bring together the results of many diverse grazing trials, and to explore the integration of cropping and grazing systems.

Plant nutrition and soil fertility

In their natural state most Australian soils are deficient in phosphorus, and many of the native plants are adapted to low levels of that element. Increase in phosphorus status, through the application of superphosphate to both crops and pastures, has been crucial to the building up of fertility and productivity. Australia uses about 5% of the world's phosphatic fertilisers, compared with only 0.5% of the nitrogenous fertilisers, and much attention has been given to optimising the methods of application and following the fate of the applied phosphorus.

Not all the responses to superphosphate are due to its phosphorus content. In 1949 sulphur deficiency was shown to occur in pastures on Southern Tableland soils, and since that time many sulphur-deficient soils have been identified in Australia. Their amelioration by superphosphate may, in some areas, accentuate selenium deficiency, a problem currently under investigation, as is the fractionation and cycling of sulphur compounds in soil. The Division also played a major part in the recognition and amelioration of molybdenum deficiency in Australia, and first demonstrated that appreciably more molybdenum is needed by legumes for symbiotic nitrogen fixation than for nitrate utilisation. We now know that molybdenum is a component not only of nitrate reductase but also of nitrogenase, a key enzyme in the process of nitrogen fixation. A recent twist to this older finding is that as the nitrogen level in soils has been built up by legume leys, increasing levels of molybdenum are required by pasture grasses and wheat crops to avoid injury by nitrates.

The impact of research by the Division of Plant Industry on pasture improvement, especially by better nutrition, has been subject to econometric analysis by Duncan¹, who found very high internal rates of return (25–80% per annum) on a number of projects.

Physiological and genetic basis of plant productivity

Under the second Chief, the cytogeneticist and plant breeder O. H. Frankel (1951–62), much more emphasis was given to longer term, more fundamental research of relevance to agriculture. Strong groups were built up in such disciplines as genetics, microbiology, plant physiology and biochemistry, and the Division grew rapidly in size and facilities.

Plant breeding continued on tobacco and several pasture plants, as well as on crops such as safflower and lucerne. A single gene controlling the relative proportions of oleic and linoleic acid was found in safflower, the forerunner to many recent programmes on selection for oil quality. Attention was also given to background research in cytogenetics, induced mutations, and quantitative and biochemical genetics, including the first identification of a biochemical mutant among higher plants. Current emphasis is on the molecular organisation of the genome and particularly on the role of repeated sequences in DNA, whose pattern can be used, for example, to identify the individual rye chromosomes in *Triticale*.

Research on symbiotic nitrogen fixation by legumes has resulted in better understanding of many aspects of the process, such as the genetic and immunological variation within the bacteria, the structure of nodules and the functioning of nitrogenase, the role of the nodule pigment leghaemoglobin, the selection of more effective strains of *Rhizobium*, and the development of better methods of seed inoculation. Whether symbiotic nitrogen fixation can be extended to crops other than legumes is a question many research groups are considering, and our Divisional research has offered some hope in this context; an effectively nodulated non-legume has been found, and rhizobial nitrogen fixation in the absence of plant cells has been shown to be possible on a defined medium. Clearly, the rhizobia possess all the genetic information for the biosynthesis of nitrogenase, and their possible association with non-legumes can be contemplated.

Photosynthesis, the process on which all agricultural productivity depends, has also been a major topic of Divisional research in recent years. It has been explored at all levels of

biological organisation, as is possible only within a large research group. How the energy-trapping and electron transport systems develop and are arranged has been one line of attack, which led to the development of a widely used method for separating the two photosystems. Attention is being given to how the components involved in energy transduction are arranged within the chloroplast membranes, to the autonomy of the chloroplasts and the role of their unique DNA, and to the pathways of carbon fixation. These need to be understood before we can hope for radical improvements in photosynthetic efficiency or for the development of other energy-yielding systems modelled on photosynthesis. The factors controlling photosynthesis at the level of the leaf or the whole plant have also been explored. Surprisingly, selection for increased crop productivity has seldom been accompanied by an increase in photosynthetic rate. In fact, this has sometimes decreased, a paradox yet to be explained. The aerodynamic processes controlling photosynthesis by plant communities have offered further perspectives on the limits to productivity. In 1971 the group most concerned with these processes became, under J. R. Philip, a separate Division of Environmental Mechanics.

Plant productivity is determined by a great many processes, any one of which may be crucial in a particular instance. Certain stages in the life cycle, such as germination or the initiation of flowering, are particularly susceptible to adverse environmental conditions, and much research has been devoted to them. Given its pan-Australian array of field stations the Division could offer a great range of natural conditions for experiment. Being uncontrolled, however, these often generated as many problems as they clarified. Impressed by the potential of phytotrons to resolve problems of climatic adaptation, Sir Otto Frankel concentrated his formidable energy on building one at Canberra. It was opened in 1962 at the completion of his tenure as Chief, and has since had a major role in much of the Division's research.

A training in pharmaceutical chemistry gave the third Chief, J. E. Falk (1963–70) an interest in the chemical control of diseases and pests of agricultural plants. He foresaw advantages in allying the microbial and biochemical research of the Division more directly with the agrochemicals industry, and established a chemotherapy group with initial support from Fisons–Geigy. Although particularly concerned with anti-fungal compounds, this group explores the relationships between structure and activity against a wide array of organisms in their search for more specific, effective and acceptable pesticides.

Crop adaptation

More attention is now being given to crops relative to pastures, in view of their increasing role in the Australian economy. Most crops are rainfed and many are severely limited in their development by water stress. Much ingenuity has been expended, since the early years of the Division, in seeking more effective resistance to or avoidance of drought stress in a variety of crops. Such work must continue because of the great potential impact of any advances that can be made, since without progress in this area the gains from other inputs into our dryland agriculture will be severely limited. One current approach is to select cereals for greater resistance to water flow through their roots, so that more water is conserved for the grain-filling stage. Another is to seek soybeans from drier regions of China than those from which the forerunners of American soybeans were drawn, in the hope of their better adaptation to low humidities.

With irrigation we move to high input agriculture and the many opportunities and environmental problems it brings. Control of pests and diseases often becomes more difficult, requiring an integration of plant breeding for resistance, modified agronomy, and the use of biological control agents together with sparing and specific pesticide use. This is part of the brief of the Divisional Cotton Research Unit at Narrabri, New South Wales. But the environment there is suitable for research on both winter and summer-growing crops, whether

irrigated or rainfed, and we expect to make it a centre for much of the research on crop adaptation.

Most crops are subject to increasingly stringent requirements for quality, particularly with respect to the content and composition of proteins and oils. Yet remarkably little is known of the nutritional, biochemical and genetic controls on protein and lipid synthesis by developing seeds. Those on storage proteins in legumes are now being actively investigated in the Division, and we hope to mount a comparable attack on the factors controlling lipid synthesis by seeds.

The Australian flora

The ecology and taxonomy of Australian native plants is now a major component of the Division's research, our concern with these plants extending beyond agriculture to their effective management as part of the national estate.

Much of the earlier ecological research concentrated on the control of weeds of pastures and crops. Together with other research on the management of native vegetation grazed by sheep, this led to an understanding of the structure and dynamics of plant communities in the semi-arid regions. Similarly, the phytochemical survey of subtropical rainforests for sources of drugs during the Second World War gave other ecologists a unique acquaintance with the great variety of rainforest communities. Yet another group of ecologists investigated the management of subalpine vegetation to maximise water catchment, for the Snowy Mountains hydroelectric scheme. Thus, the Division built up ecological expertise ranging from alpine to tropical and from arid to high rainfall environments. Currently this finds many outlets, as in research on the revegetation of mine dumps, on the use of native grasses in cityscapes, and on the management of national parks and reserves. The effects of fire and of fire regimes on several types of vegetation are being analysed because fire has long been used by the aborigines and by graziers, foresters and National Park rangers to manage a variety of plant communities, without adequate knowledge of its long term effects.

Inadequate taxonomic knowledge of native plant species also remains a bottleneck to progress. New genera are still being found, and even new families, such as the recently described Idiospermaceae. The Division set up a herbarium in its early years, primarily to assist the identification of weeds and of poisonous or introduced plants. It now has a representative collection of the Australian and New Guinea flora, recently gazetted as the Herbarium Australiense, and Nancy Burbidge and other taxonomic staff are taking the initial steps towards preparation of a revised Australian Flora, a task which will require many years for its completion.

Past and future

This brief account of the CSIRO Division of Plant Industry has emphasised the evolution of its role and research over the past 50 years. Its course has been shaped by external influences such as depression and war, by the perspectives of successive Chiefs, and by the enthusiasms and findings of its research staff over the years. As the Division has grown it has given birth to four daughter Divisions (Land Research, 1950; Tropical Pastures, 1959; Environmental Mechanics, 1971; and Land Resources Management, 1973), whose dowries have included major areas of research and most of the field stations. At no time has the Division lacked challenging and important problems to be tackled, and it has been able to approach these at all levels from the most fundamental to the applied. This capacity to mount a varied and profound attack on problems of significance to Australian agriculture has given the Division a sense of both scientific and practical achievement, as well as many opportunities for fruitful interaction between researchers from the various disciplines and levels of analysis. It is a feature we cherish, and which we hope can be preserved through the next 50 years.

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Synthetic polymer research in CSIRO

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Polymer research began early in the development of CSIRO, and is now spread through several divisions. The results have a wide range of industrial applications.

ALTHOUGH no single research division of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) is devoted solely to polymer or macromolecular science, a considerable amount of research is done on synthetic polymers in the total organisation. Research has been carried out in the division where the need has arisen and coordination and cooperation between the various divisions and the scientists concerned is organised through a series of regular interdivisional colloquia. The subjects studied vary widely over many fields according to the scientific and technological content required by each problem. In this article the examples have been chosen to illustrate the way in which applied research is tackled within CSIRO, and the relevance of this research to the Australian scene and to science generally.

Studies on synthetic polymers have been initiated to investigate problems as disparate as fire resistant construction in building, modification of the properties of woollen fabrics (shrink-proofing and permanent press), water treatment, boiler scale prevention and weather resistant paints.

The major research topics are listed in Table 1, which shows that polymer research started early in the history of CSIRO with investigations by the primary industry divisions into natural polymers as an adjunct to their main projects. Synthetic polymer research began in earnest with the establishment of the Division of Industrial Chemistry in 1941 when CSIRO moved into research for secondary industry. The projects at that time were dictated by war-time requirements; for example, investigations into the phenol/formaldehyde reaction were undertaken with the aim of improving the production of variable density wooden aircraft propellers.

It was at this early stage that the pattern of in-depth investigation of practical problems, followed by application of the fundamental knowledge gained to formulate solutions, was established. Often the knowledge gained led to the solution of additional problems. In every case the scientists involved were encouraged to follow the project through to the practical solution of the original problem. This is still the approach used.

Novel polymer chemistry

Two examples — polyesterification and water-dilutable isocyanate polymers—have been chosen to illustrate research in novel polymer chemistry.

Polyesterification was investigated by what is now the Polymer Group of the Division of Applied Organic Chemistry. In 1964 the group was approached by a local paint company making alkyd resins (fatty-acid-modified polyesters) because the properties of their resin batches did not accord with those expected from theoretical calculations based on the initial reactant mixture and the degree of reaction. Contrary to popular belief, alkyd resins prepared by different methods from the same raw material composition did not always have the same solution or film properties. It appeared that Flory's theories relating to

equal reactivity of functional groups irrespective of the size of the molecule and rapid interchange of ester groups did not apply in many polyesterifications. Considerable evidence¹ was accumulated during the research to demonstrate that the properties of an alkyd were related to the method used in its manufacture, the order in which the monomeric components were added and the composition of any intermediate products formed, for example by glycerolysis of a vegetable oil. This demonstrated that ester interchange reactions were slow compared with polyesterification in the conditions used to make commercial alkyd resins.

The study also established that not all the residual hydroxyl groups in an alkyd are available for reaction in the polyesterification process or for reaction with compounds of low molecular weight, for example in acetylation, phthalylation, or cross linking¹. The availability of the hydroxyl groups was limited particularly in highly condensed (branched) systems with an average functionality greater than two. Infrared studies of such alkyds acetylated by normal methods using extended reaction times showed an absorption band at $3,550\text{ cm}^{-1}$ indicative of bonded hydroxyl groups.

The problem of lack of reactivity of some hydroxyl groups in nonlinear polyesters was resolved with the discovery that many alkyd resins contain dispersed particles $0.05\text{--}1.0\text{ }\mu\text{m}$ in diameter which, since they consist of molecules with infinite networks, have been termed microgel particles². It was the hydroxyl groups within these particles that were bonded and unreactive. Thus alkyds which contain microgel do not fulfil one of the basic conditions explicitly cited by Flory—that the polymer molecules are present in a homogenous reaction mixture.

An explanation was thus found for the apparent conflict with Flory's theories. Microgel formation may occur much earlier in the reaction than physical gelation; in fact, microgel has been found in a formulation in which gelation was not predicted². It follows that, in some formulations at least, some molecules enter an environment in which they react much faster than others. In such cases, Flory's principle of "equal reactivity for equal functional groups" does not apply.

This evidence, plus a consideration of the change in chemical composition and consequent changes in viscosity and polarity of the reaction medium during polyesterification, led to the conclusion that kinetic analysis of the whole course of this reaction was of doubtful validity and was inappropriate in many nonlinear formulations. It was possible, however, to study the kinetics of polyesterification in linear systems in conditions equivalent to the later stages of polyester formation where the polarity of the solvent (polyester) did not change appreciably and the concentration of reactants was low enough for sensible kinetics. This study³ supported Flory's conclusion that simple esterifications and polyesterifications are of the same kinetic order. In the absence of added catalyst they both follow third-order kinetics with rate $\alpha[\text{RCOOH}]^2[\text{ROH}]$. These experiments with linear polyesters also provided support for the concept that the reactivity of a functional group is independent of the size of the molecule to which it is attached since even when the polyesterifications were taken to a higher degree of conversion (that is high molecular weight) than Flory's studies, the kinetic order still applied.

After the establishment of the kinetic orders of the catalysed and uncatalysed reactions a mechanism consistent with all the results was deduced. The novel concept was that the rate controlling step was the reaction of a $[\text{RCOO}^- \cdots \text{RCOOH}_2^+]$ ion pair with ROH (ref. 3).

The clarification of the kinetics and mechanism of esterification and polyesterification reactions and the presence of microgel in alkyds was of considerable scientific value. In addition, the demonstration of microgel formation and the explanation of the lack of reactivity of some hydroxyl groups in alkyd resin was of great practical importance. Because alkyd resin solutions which contain microgel have the high molecular weight polymer present in dispersion these resins offer advantages in a paint over an alkyd in which the polymer is in true solution. Foremost among these are lower viscosity at the same solids content and faster drying in air drying formulations².

These advantages were such that the paint company which had presented the original problem set about deliberately to make alkyd resins with high microgel content and now have a patented method of producing a rapidly drying alkyd paint rich in microgel. In addition, the knowledge gained during this study, and subsequently published, made possible greater understanding of alkyd resin manufacture and consequent improvement in quality of the alkyd paints made by all manufacturers.

Another fundamental study of polymer chemistry which is finding important practical use was the investigation into novel isocyanates used initially for shrinkproofing, and recently for conferring 'permanent press' qualities on woollen garments. This investigation and its practical application was performed by the Divisions of Protein Chemistry and Textile Industry of CSIRO's Wool Research Laboratories.

The surface of a wool fibre is covered with scales oriented in one direction, and so in a textile the wool fibre can move more readily in one direction. When the textile is washed, the relative movement draws the fibres together and can also cause the development of slack sections. The two effects combined produce felting, shrinkage and distortion of garments. Relative movement between fibres can be prevented by interfibre bonding or by masking of the scales on the surface of the fibres. The component divisions of the Wool Research Laboratories have been working for many years on this problem and have developed a number of useful processes. The method most widely used to

prevent shrinkage is to apply partly polymerised materials (prepolymers) either from solution in organic solvents, or preferably from aqueous systems followed by curing of the coated fibres or fabric, to complete the formation of an insoluble polymeric film.

Isocyanate prepolymers are used in CSIRO's current permanent press process⁴ which has been in wide use in Australia since 1971, especially for service uniforms. But because of the reactivity of the isocyanate groups with water the prepolymers must be applied to finished garments from organic solvents in anhydrous conditions in a modified drycleaning machine and this has limited the commercial application of the process. In an attempt to develop systems which could be applied from water, methods were sought for masking the isocyanate groups, so that they are stable in water but still able to undergo subsequent polymerisation. Research starting from some early German work on the reaction of sodium bisulphite with simple model isocyanates in water demonstrated that these groups in isocyanate prepolymers can be masked by forming bisulphite adducts if alcohols are used as solvent⁵. The products are water soluble and stable to storage in aqueous solution. After the aqueous solution of the polymer is applied to wool the coating is cured as a result of the polymerisation of the isocyanate entity. This work has led to the Sirolan BAP⁶ process which is extremely simple; the polymers are applied by padding using conventional textile equipment and then cured by drying. The process has been thoroughly proved in extensive industrial trials carried out in collaboration with the Australian Wool Corporation and the New Zealand Wool Board, and is expected to be used widely to impart machine washability to knitted fabrics, thus increasing the market acceptability of wool, one of Australia's most important export commodities.

Polymers for water treatment

In the group concerned with water purification research in what is now the Division of Chemical Technology, interest in the area of carbon and ion exchange led to the proposal of electrically regenerable ion-exchange systems based on semiconducting polymers. This followed a detailed study of the chemistry of carbon surfaces, in which it was shown that many carbons are organic polymers containing functional groups. The principle of electrical regeneration was shown to be sound, but space charge effects were observed which made it improbable that high exchange capacities

Table 1 Polymer research topics in CSIRO

Division	Topic
Forest Products (1927-71)	Structure of eucalypt wood; hardwood pulping processes; lignin; polysaccharides
Industrial Chemistry (1941-58)	Phenol/formaldehyde plastics; kinetics of the phenol/formaldehyde reaction; tannin-formaldehyde adhesives; reinforcing fillers for rubber; carbon black chemistry; conducting polymers
Physical Chemistry (1958-66) Applied Chemistry (1966-73) Chemical Technology (1974-)	Thermally regenerable ion-exchange resins, polymers for water treatment; magnetic filter aids.
Applied Mineralogy (1964-70) Applied Chemistry (1970-73) Applied Organic Chemistry (1974-)	Clay minerals and polymerisation; polyesterification; treated fillers for plastics; controlled release coatings for ruminant foods; cyclopolymerisation; synthetic antigens; chelating polymers
Protein Chemistry Textile Physics Textile Industry (1950-)	Structure of wool and wool proteins; polymeric coatings for wool; mechanical properties of fibres and fibre assemblies; polymeric coatings for leather
Food Research	Gas permeability of polymer films
Chemical Engineering	Rheology of polymer solutions and melts; polymers for boiler scale prevention
Building Research	Plastics in building; weathering of plastics and paints; fire resistance of plastics; mechanical properties of wood; tannin/formaldehyde adhesives

could be achieved when a large number of sites were present.

The realisation that heat would regenerate the resin more readily in certain systems has led to a new concept in ion exchange. Sirotherm (ICI Australia) resins were developed around the concept of regeneration with hot water rather than chemicals and their use has now reached the commercial stage with a plant built by ICI Australia Ltd to desalt $600 \text{ m}^3 \text{ d}^{-1}$ of low salinity water in Adelaide. A vigorous polymer synthesis programme has been maintained during the development stage, with the preparation of many resins of the weak electrolyte type. The acidic resins used are based on acrylic or methacrylic acid; a wide range of basic resins such as *N*-substituted polyvinylbenzylamines, polyvinylamines, polyaminoethylmethacrylates and polyallyl amines have received attention. In addition, there has been the need to utilise microparticles to achieve sufficiently rapid rates of reaction. Ways of forming these into easily handled composite particles by various means, using polymeric multiphase systems, have been investigated.

When the commercial production of Sirotherm resins was first considered, it was realised that expensive initiator systems such as ultraviolet or γ radiation used during initial development needed to be avoided. The Division of Applied Organic Chemistry developed chemical initiation systems for the possible basic components. During this study it was shown that in the polymerisation of diallyl amines the ring size formed during the cyclopolymerisation was important in determining the properties of the resin. In an intensive study the polymers were examined by ^{13}C nuclear magnetic resonance to determine the presence and proportions of five-membered and six-membered ring structures and unchanged allyl residues. The structures of the reacting radicals and polymers were determined by electron paramagnetic resonance both on polymerising systems and model compounds. This study has shown that polymerisation of substituted diallyl amines gave linear saturated molecules containing predominantly five-membered ring structures⁷ whereas earlier workers in the field suggested that six-membered rings were formed. It has also been shown that kinetic and steric factors played a greater part in determining polymer structure than did thermodynamic stability. The knowledge gained in this study has led to simple methods of control of ring size and basicity during the production of basic ion-exchange resins from substituted allyl amines.

Microparticles were needed in the thermally regenerable ion-exchange process to ensure rapid rates of ion-exchange. The concept of magnetically flocculating microparticles to ease the difficulty of handling them has led to novel engineering procedures for standard ion-exchange processes. The magnetic polymers are prepared by embedding iron oxide in the resin particle. Both homogenous and shell materials have been prepared which can contain carboxylic acid, sulphonic acid, amino, or quaternary ammonium groups. Magnetic polymers devoid of functional groups have been tested successfully, in hydrophilic forms as magnetic filter aids. The high voidage of the flocculated magnetic polymers makes for an ideal filter bed, which has the advantage that it is easily recoverable and can be used over many cycles. Hydrophobic versions have been used in the collection of oil spills, with the oil being absorbed into the voids in amounts up to 20 times the volume of the magnetic polymer itself. Many applications are envisaged for these magnetic polymers, particularly but not exclusively in water treatment and recovery.

Polymers for biological systems

A developing area of polymer research in CSIRO is the use of polymers *in vivo* for increasing the productivity of animals and for use in human medicine. The examples

described here—controlled release and synthetic antigens—are directed towards increased wool and meat production.

It was found in the Division of Animal Physiology (now the Division of Animal Production) that certain amino acids give enhanced wool growth when injected into the abomasum (fourth stomach) of a sheep. They have no effect, however, when administered orally, probably because they are microbiologically degraded in the sheep's rumen⁸. A basic polymer which was insoluble in the conditions normally experienced in the rumen (24 h, pH 7, 37 °C) but soluble within 1 h in the abomasum (pH 3) was designed and synthesised. This polymer can be used to coat amino acids or any other food or drug to give protection from degradation in the rumen of any ruminant animal. Polymer synthesis including a fundamental study of copolymerisation of monomers with basic functional groups, methods of coating amino acids with the polymer, and methods of administration of the coated material to sheep were all investigated and a workable system was devised and licensed to an international company⁹. Coated feeds should shortly be marketed and, because of a shift in markets during the course of the project, are expected to find a major application in feedlot production of beef as well as for supplementary feeding of sheep to enhance wool growth.

Increased wool and meat production by the cutting of losses due to infertility and pest attack is the result expected from the current work on synthetic antigens designed to generate antibodies which bind non-protein molecules. There are two immediate uses for this work; first in the radioimmunoassay of ecdysones (arthropod moulting hormones) in connection with work on pest control, and second for the immunisation of sheep against "clover infertility disease" which is a major economic problem in the Australian sheep industry.

Moulting is an essential process in the development of arthropods and interference with this process, either by causing premature moulting or by delaying moulting would cause death and thus could be used to control insect, tick or nematode populations at an early stage of development. Successful use of this control method, however, can only come after a knowledge of the natural concentrations of hormones present at the various stages of arthropod development is obtained. To do this requires the measurement of minute quantities of ecdysones, and radioimmunoassay is by far the most convenient technique.

Synthetic antigens had been used to generate antibodies for the radioimmunoassay of ecdysones previously, but the ecdysones had been linked to bovine serum albumin by the functional groups of the tetracyclic body of the molecule. As a consequence the ecdysone was partly masked by the protein carrier and the resultant antibodies were unselective and unreliable. By masking the functional groups of the tetracyclic ring and attaching the ecdysone through a side chain to the high molecular weight protein thyroglobulin, an antigen which produces antibodies in the rabbit specific to the tetracyclic structure of the ecdysone molecule was produced. This antibody can be used for radioimmunoassay of various ecdysones with the same tetracyclic structure, but is sensitive to small changes in the tetracyclic part of the ecdysone, and consequently gives very reliable results.

The incidence of "clover infertility disease" in sheep is correlated with the presence in pasture plants of the phyto-oestrogen, formononetin, which is metabolised to equol in the rumen of the sheep¹⁰. It is now known that it is equol which is responsible for the oestrogenic effect of ingesting clover. For this reason a programme to try to immunise sheep against equol was initiated. In the work so far, derivatives of equol conjugated, at a position remote from the main functional groups, to bovine serum albumin have caused the formation of high levels of antibodies specific to equol in both rabbits and sheep. The results have been

sufficient to justify an extensive field trial of immunisation with these synthetic antigens on sheep in West Australia where the problem is greatest.

A number of proteins have been used successfully as carriers in the synthetic antigen conjugates but they all suffer from problems of, for example, hydrolytic stability. Work is in progress to use synthetic polymers as carriers in these antigens. The control available over polymer composition and molecular size as well as greater stability should enable the current problems with protein conjugates to be overcome and should extend the use of synthetic antigens into other areas.

As illustrated in the examples discussed here, CSIRO polymer research is operating in many areas and at various scientific levels. The overall philosophy of this research

which is similar to the approach used in other fields, is that the problem under investigation should be studied in sufficient depth to gain a proper understanding of the principles of the process. The value of this approach has been proved in many areas where not only the original problem was solved, but new lines of investigation and new applications for the knowledge gained have resulted.

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Australian journals of scientific research

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Since the Second World War the growth of scientific research in Australia has led to the creation of a group of nine national journals. CSIRO has had a major part in the creation, funding and running of these journals.

AUSTRALIAN scientists had few problems in finding suitable journals in which to place their research papers up to 1939. Output was small and it was easily absorbed by existing journals—mainly British and American and those few publications which had grown up in Australia during and after the First World War. The latter were primarily concerned with agricultural, medical and veterinary science. The best known are the *Medical Journal of Australia* (launched in 1914), the *Australian Journal of Experimental Biology and Medical Sciences* (1924), *Australian Veterinary Journal* (1925), the *Journal of the Australian Institute of Agricultural Science* (1934) and *Australian Forestry* (1936).

The Second World War was responsible for an enormous expansion in scientific activity in Australia. The Council for Scientific and Industrial Research (CSIR) with a total staff of 400 in 1937 had grown to 2,000 by 1946. It was evident to David Rivett (the wartime Chief Executive Officer of CSIR) and to some of his senior scientists that the traditional methods of disseminating research results were inappropriate to post-war conditions. Papers which fell outside the interests of existing journals tended to be published as CSIR Bulletins. Their content was, however, so diverse that distribution had become a problem to CSIR and their classification a headache to the libraries which received them. In 1947 CSIR, supported by the Australian National Research Council (ANRC), decided to create a national journal, the *Australian Journal of Scientific Research*. It was to be divided for convenience into two sections: Series A for the physical sciences and Series B for the biological sciences.

Dr N. S. Noble was appointed Editor by the two bodies and an Editorial Board comprising Professors W. J. Dakin, E. J. Hartung, L. H. Martin and J. G. Wood was set up. The first journals appeared in 1948 on a quarterly basis. In this first year 36 papers were published in Series A and 26 in Series B. The Editor acted as both Executive Officer and Chairman of the Editorial Board—a dual responsibility which was difficult to discharge, but which was later resolved when an independent Chairman was appointed by the sponsoring bodies.

The Editorial Board's functions were defined as follows: first, 'To take responsibility for acceptance and rejection of papers submitted to the *Australian Journal of Scientific Research*.' Second, 'To advise the Editor on matters of editorial policy.' Third, 'To advise CSIR and ANRC on matters affecting the publication of the *Australian Journal of Scientific Research*.'

All papers were refereed and contributions were accepted from any scientist regardless of institutional affiliation whether university, state or Australian instrumentality or industry. Originally about 60% of the submissions came from within CSIR—a proportion that has steadily decreased over the years to 25%.

By 1949 there was already some evidence of the problems which beset nationally sponsored journals. The *Australian Journal of Scientific Research* was tending to become an omnibus publication which even then had little appeal to the fast-fragmenting scientific community. Key¹ representing the views of CSIRO (CSIR changed its name to the Commonwealth Scientific and Industrial Research Organisation in 1949) scientists said that this led to "a general undervaluation of Australia's contribution to science". A part answer to this was the creation of three more journals: *Australian Journal of Agricultural Research*, *Australian Journal of Applied Science* and *Australian Journal of Marine and Freshwater Research*. These were sponsored entirely by CSIRO as the ANRC was not invited to collaborate in their establishment or control and the Editorial Board of the *Australian Journal of Scientific Research* was not concerned with them in any way.

An Editorial Advisory Committee, however, was appointed for each journal by the Executive of CSIRO. It was their responsibility to recommend acceptance or rejection of papers submitted for publication. Although printing difficulties and shortages of paper at the time made it impossible for the publisher to solicit contributions from outside CSIRO, papers were never rejected on those grounds alone.

Criticism of the nonspecific titles of three of the journals continued. How was it possible to decide whether a paper on plant-soil relationships was agricultural, applied or just scientific research?

By 1952 the Editorial Board decided that enough material of good quality was coming forward for Series A to be divided into two parts, namely the *Australian Journal of Chemistry* and the *Australian Journal of Physics*. The Executive of CSIRO decided to make the change in 1953 and invited the Royal Australian Chemical Institute to collaborate in the publication

Table 1 Summary of statistics for 1975

<i>Australian Journal of</i> (issues)	Pages published	Published papers	Rejected papers	Lapsed papers
<i>Agricultural Research</i> (6)	1,079	107	25	19
<i>Biological Sciences</i> (5)	573	60	22	7
<i>Botany</i> (6)	973	62	13	2
<i>Chemistry</i> (12)	2,744	331*	115	16
<i>Marine and Freshwater Research</i> (3)	428	36†	7	1
<i>Physics</i> (6)	1,043‡	80‡	38	7
<i>Plant Physiology</i> (4)	668	58§	15	6
<i>Soil Research</i> (2)	238	21¶	13	2
<i>Zoology</i> (4)	842	46	12	8
Total (48)	8,588	801	260	68

*Includes 87 short communications.

†Includes 11 short communications.

‡Includes 5 short communications and 5 supplements, 328 pages.

§Includes 6 short communications.

¶Includes 1 short communication.

||Includes 2 supplements, 234 pages.

of the *Australian Journal of Chemistry* and the Institute of Physics (Australian Branch) in the *Australian Journal of Physics*. The Australian Institute of Agricultural Science and the Australian Veterinary Association had previously been invited to collaborate with the *Australian Journal of Agricultural Research*. As Series B could no longer continue without its partner, the journal was renamed the *Australian Journal of Biological Sciences*. In an attempt to answer all the previous criticisms the Executive of CSIRO established two further journals, the *Australian Journal of Botany* and the *Australian Journal of Zoology*, to provide outlets for the more descriptive papers in these disciplines.

Extension of functions of the Board

The ANRC agreed to collaborate with CSIRO in sponsoring all the eight research journals which were to be published from 1953 onwards. The Editorial Board's function was amended to enable it to advise CSIRO and ANRC and assist the Editor in the publication of the eight journals. Editorial Advisory Committees were appointed for each journal and members of the Editorial Board were asked to serve on appropriate advisory committees.

The Australian Academy of Science

In 1957, the Australian Academy of Science agreed to fill the position left vacant by the disbandment of the ANRC in 1955 so that it now acts as a joint sponsor of the journals with CSIRO. A Board of Standards was set up to control editorial policy. The Board comprises a Chairman, a nominee of the Academy, a nominee of CSIRO, the Chairman of the Editorial

Advisory Committees and the Editor-in-Chief. Its functions are spelled out in the Academy's Yearbook².

Subsequent developments

In 1963 the *Australian Journal of Soil Research* was added to the series and one year later the *Australian Journal of Applied Science* ceased publication through lack of support. Dr Noble, who had seen all these developments and who had been responsible for the quality of the textual matter and high standards of presentation, retired in 1963 to be succeeded by Mr A. E. Scott who had been his senior editor. The journals grew steadily in stature under Mr Scott's stewardship until his retirement in 1971. Following several years of discussion with biological scientists, the Board of Standards recommended the creation of the *Australian Journal of Plant Physiology* in 1974.

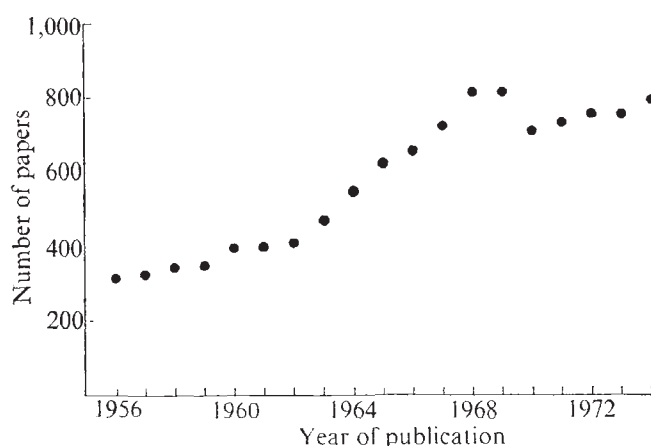
Thus there are now nine journals in the series, whose current vigour can best be summarised by the publication statistics shown in Table 1 and the pattern of growth shown in Fig. 1. The number of papers published in 1975 was 500 more than in 1953 and 1,250 papers are now submitted annually. In the past 18 months there has been an abnormal increase in their number, partly because of difficulties being experienced in publishing outside Australia. Page charges of up to \$100 are now being quoted by some US publishers and many journals are having to decline foreign papers.

Problems associated with national journals

It has always been difficult to persuade scientists in countries with small populations to support their local publishers. The problems have been discussed in some detail by Gregory³ and Lim Huck Tee⁴. Australian scientists were no different, particularly in the first few years. Many considered that their work might be suspect if it were published in an Australian journal, while others rationalised their distrust by pointing to inadequate distribution of the journals and lack of awareness of their existence among scientists overseas. Even the titles of the journals have been criticised at times on the grounds that they represent an insular attitude to research, although it is difficult to see how one can differentiate between, say, one journal of botany and another without some adjectival label. Somehow names like *Helvetica Chimica Acta* and *J. Am. Chem. Soc.* have not prevented journals associated with particular nations or societies from being considered international in every sense.

The commonest complaint still heard is that specialists must publish only in specialist journals. The difficulty is that national journals tend to cover a broad spectrum of topics unless there is an active group of scientists of world renown working in the country in question who are able to attract contributions from overseas. The *Australian Journal of Plant Physiology* seems to have achieved this happy state in less than two years although it must be remembered that the *Australian Journal of Biological*

Fig. 1 Number of papers published annually since 1956.



Sciences published first-class papers in plant physiology for many years before the new specialist journal appeared. Specialisation in a national journal can also have its drawbacks. The *Australian Journal of Physics* has had a reputation (rather unfairly one feels) for specialising in radiophysics and high energy physics. This has persuaded some physicists that the editors are not interested in publishing in such areas as solid-state physics or optical astronomy. These prejudices can be broken down only slowly with the help of the advisory committees and a welcoming editorial attitude. On the other hand, acceptance of substandard papers in 'new' disciplines will certainly not achieve the required result.

The efficiency of secondary publication services these days puts any journal in the public eye more effectively than ever before. An author's search for visibility through large circulations is now no longer necessary. The general decline in the world's postal services has ensured that a scientific contribution from Australia is noted in current awareness documents like *Current Contents* long before the journal itself reaches the ultimate reader. Requests for reprints often arrive before the reprints have left the publisher. It is, of course, the publisher's responsibility to ensure that his journals are sent promptly to the leading abstracting organisations and that they are laid out in the most appropriate way for easy incorporation into the appropriate data bases and secondary journals. The Australian Journals of Scientific Research were redesigned in 1974 with this in mind. Each contribution is now headed by full bibliographical details and an indicative abstract, while information that is irrelevant for retrieval purposes has been relegated to footnotes or to the end of articles.

Recent studies of citations indicate that the Australian journals rank well in their various categories. Thus the *Australian Journal of Biological Sciences* ranked thirtieth in Garfield's analysis of most frequently cited botany journals⁵ (this was before the *Australian Journal of Plant Physiology* appeared).

The *Australian Journal of Physics* is creditably placed among the leading astronomy journals even though it carries only a small proportion of radiophysics papers these days. In agriculture, the *Australian Journal of Agricultural Research* is listed thirty-eighth in the top 100 journals included in a recent Science Citation Index analysis⁶.

Advisory committees

Each journal in the series has a separate editorial advisory committee. The chairman of each committee is appointed jointly by the Academy of Science and the Executive of CSIRO and automatically becomes a member of the Board of Standards.

Committee members are chosen by the Board of Standards with an eye to ensuring as wide a coverage as possible of the topics likely to be found in the journal. It is their responsibility to keep a watching brief on the standards of the papers and to act as arbiters when referees fail to agree on the worth of any particular submission. They are also available to help the editors choose referees. Although the committee of each journal normally meets only once a year, it is continually at work

Table 2 Overseas recipients of Australian journals

USA	2,560	France	228
UK and Eire	1,052	South Africa	196
Canada	514	Italy	170
Japan	438	Netherlands	154
India	425	Pakistan	151
USSR	355	Indonesia	145
Germany (all)	349	Malaysia	116
New Zealand	230		

encouraging people to submit papers to the journal, watching for new areas of research that would be of interest to the journal and doing those many vital and unpaid tasks that are the lot of any publishing committee. There are currently sixty-two scientists employed in such activities for the nine journals.

Referees

The standard of any scientific journal ultimately rests on the quality of its refereeing system. All papers submitted to the journals are refereed thoroughly by independent authorities selected by the editors and their advisory committees. Naturally, most referees are chosen from research centres in Australia and New Zealand, but there has been, over the past four years, a much greater dependence on referees in Europe and North America. At the same time, the total number of referees used has expanded dramatically. The use of such overseas expertise has had a marked effect on the quality of the papers submitted from outside the immediate catchment area. The newest of the journals, the *Australian Journal of Plant Physiology*, has already attracted the attention of authors from Japan, Germany, the United States and Israel.

The publishing office

Production of the set of national journals in East Melbourne is unique. Even the Canadian journals are not compiled in such a closely integrated manner and the Australian journals have the added advantage of being printed by the publisher. The editors—each specialists in their own right—are in daily contact with each other. If a mathematical problem arises in a biological paper there is immediate help at hand from say one of the physics editors or if a biochemical paper contains an unusual compound its structure or name can be checked by a specialist in chemical nomenclature.

Production methods are continually being improved by the use of modern printing techniques, the inclusion of computer-generated tabular matter and better methods of binding and packaging. The only backward area—and it seems to be the one that defeats all publishers today—is cheap and swift distribution of the final product to the readers. But every international publisher believes that his own postal system is the worst in the world.

Relations with the scientific community

The close relations with the Royal Australian Chemical Institute, the Australian Institute of Physics, the Australian Institute

Table 3 Distribution of publishing time from receipt of manuscript

Australian Journal of	Elapsed time (months)											Total number of papers
	3	4	5	6	7	8	9	10	11	12	> 12	
<i>Chemistry</i>	5	50	115	100	27	19	10	1	3	2	1	333
<i>Biological Sciences</i>	5	8	14	40	28	14	6	9	2		4	130
<i>Agricultural Research</i>		4	14	30	17	12	2	4	4	2	5	94
<i>Physics</i>		2	11	17	18	13	10	8	1	1	3	84
<i>Zoology</i>	8	6	4	7	3	1	1					30
<i>Botany</i>	1	2	5	2	3	7	2	1			1	24
<i>Marine and Freshwater Research</i>	3		3		1		1	1	1		1	11
<i>Soil Research</i>		1	1	1	1	2	2		3			11
Total	22	73	167	197	98	68	34	24	14	5	15	717

of Agricultural Science, the Australian Veterinary Association and the Australian Society of Soil Science have been mentioned already. More strenuous efforts have been made recently to encourage members of other scientific societies to consider the journals as natural homes for their own research papers. These overtures are being gratefully received at a time when most societies are finding it increasingly difficult to publish more than a simple news bulletin because of the soaring costs of printing and administration. Moreover, many journals published in other countries are either resorting to high page charges or otherwise discouraging Australian authors by restricting the length of papers or increasing the period that a paper remains 'in press'.

Concessional subscription rates are now offered to the members of sixteen scientific societies in Australia at prices which represent little more than the run-on cost of the journals plus an allowance for postage and handling. The scheme has been well received by the societies and their members.

It has been mentioned already that support for the journals has been canvassed among all scientists in Australia and not merely among CSIRO staff. Now half the published papers come from Australian universities, one-quarter from CSIRO and the rest from other institutions in Australia and abroad.

In 1975 some 48 issues of the various journals together with seven supplements were published. Over 800 papers were published in 8,588 pages and 14,100 sets of the various journals were distributed in more than 100 countries. The main recipients of the journals outside Australia are listed in Table 2.

The most recent analysis of publication times is shown in Table 3. The median time from receipt of manuscript to publication is six months based on a total sample of 717 papers taken from eight of the journals. The *Australian Journal of Chemistry* has the best record (5 months) because it is the only publication in the series that is published monthly. All the others are published less frequently (Table 1). It takes about three months from acceptance for a paper to appear in most of the journals. This figure is fairly constant as a result of the tight schedules kept by CSIRO's printers. Refereeing is responsible for the greatest part of the variation in publication times, particularly when manuscripts have to travel long distances. It is worth

noting, however, that referees in Europe and the USA generally do everything they can to speed up the refereeing process by returning their reports promptly.

The future

The scientific journal in its present form will not disappear overnight. Too many conservative forces are at work for this to happen. Agreement between authors, publishers, printers, librarians and readers is necessary before a more efficient system of publication, storage and dissemination of primary research results is likely to be accepted. But our problems are no different from those of others engaged in generating scientific knowledge. How to do more with shrinking resources in real terms? Luckily, the tremendous increase in output during the past few years seems to be slowing down, but this alone will not save the publisher from the effects of inflation on everything he does. More use must be made of storage centres for extraneous data not needed by the majority of readers; substandard submissions must be ruthlessly rejected or sent back to authors for restyling; cheaper and faster methods of production must be sought, second thoughts by either editors or authors cannot be tolerated at the proof stage; indeed, perhaps the time has come when proofs should not be sent to authors except in special circumstances, and parts of the journal may be issued in microform only. CSIRO's computer with its microfilm output equipment (a COMp 80 phototypesetter) is now being used to produce camera-ready copy for the supplements to the *Australian Journal of Physics*. The same data can be published in microfiche with little more than a change of camera. Experiments have shown that it is feasible to set complex mathematical text more economically by computer than by anything other than strike-on systems such as the IBM composer. Our aim, however, remains to produce text of high quality with first class typography in the most appropriate format.

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articles

On the mechanisms of lunar regolith glass particle formation

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The discovery of many particles of glass in the lunar regolith has resulted in various hypotheses for their origin¹. Based on previously unpublished work², we here give an analysis of the principal mechanisms of their formation and evolution.

FIRST, we show that the condensation of vapour created by the impact of a meteorite does not make a noticeable contribu-

tion to particle formation but spraying does. For this we consider the size distribution of lunar glasses³⁻⁵, which has a bimodal character, with a sharp left maximum in the range of 0.1-10 μm and a weak right maximum in the range of 100-500 μm (see Fig. 1) and compare it with the distribution function of meteor material. The linear dimensions (r) of condensate particles are proportional to those of the initial meteorite (R) (ref. 7): $R = Kr$ and $K \approx 10^3$. This gives a characteristic size of condensed particles of $\sim (1-5) \times 10^{-3} \mu\text{m}$, that is, almost atomic size. The

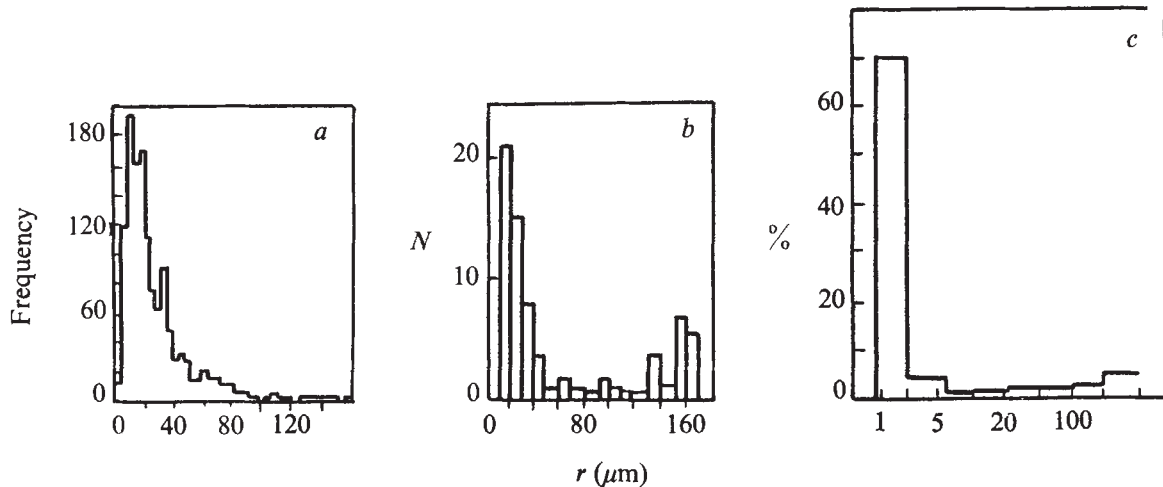


Fig. 1 The size distribution of lunar glasses. Data in *a* are taken from ref. 5, in *b* from ref. 4 and in *c* from ref. 3.

spraying of the impact-melted material gives an analogous coefficient $K' \approx 10$, leading to characteristic sizes $r \approx 0.1-1 \mu\text{m}$ (ref. 2). As far as we know the finest-grained glass particles have been measured in detail only in ref. 6, and the data agree with this result.

The characteristic particle size (d) can be estimated in another way from the cohesive characteristics of lunar regolith²

$$d \approx 2r_m(\sigma_i/\sigma_m)^{1/2}$$

where r_m is the intermolecular interaction distance, $\sim 10^{-7} \text{ cm}$, σ_i is the individual particle strength, $\sim 10^9 \text{ dyne cm}^{-2}$, and σ_m is the macroscopic shear strength of lunar regolith. Taking $\sigma_m = 5 \times 10^3 - 5 \times 10^4 \text{ dyne cm}^{-2}$ gives $d \approx (0.5-2) \times 10^{-1} \mu\text{m}$ (ref. 8). This value partially overlaps the previous estimate and therefore corroborates the idea that the spraying mechanism during meteorite impact is predominant in the formation of small glass particles.

The large particles of the right mode, $\sim 100-500 \mu\text{m}$, can form only as a result of slow endogenic processes and thus should be 'older' than the small ones.

The spraying mechanism

Regular particles are formed from the decay of irregular jets of melted material by the interplay of inertia and surface tension. Balancing these forces brings about some characteristic maximum stability size r_{st} that depends on characteristic process rates. The left and right peaks in particle size distribution are related to the high velocity (meteorite) and low velocity (endogenic) processes, respectively. Indeed, if $(\bar{v}^2)^{1/2}$ is the r.m.s. internal velocity of a liquid particle and α is the coefficient of surface tension, then we get

$$\frac{\bar{v}^2}{2} \approx \frac{\alpha}{r_{st}}$$

(ρ is the density of the liquid). It would be natural to assume that $(\bar{v}^2)^{1/2}$ is related to the velocity, u , of a typical particle, size l , so that the ratio $(\bar{v}^2)^{1/2} \approx ur/l$. Thus it follows that

$$r_{st} \approx \frac{l}{u} \left(\frac{2\alpha}{\rho} \right)^{3/2}$$

One can easily calculate that $r_{st} < 10 \mu\text{m}$ for the velocities and dimensions characteristic of meteorite impacts and all the particles generated in such a way are in the region of the left mode.

The shapes of the glass beads

The relationship between the elongated (ellipsoidal dumb-bell) and spherical shapes of particles will be considered next. The drops will vibrate (if energy of their internal movements is not sufficient for decay) as

$$X = X_0 \exp(-\gamma t) \sin(\omega t + \delta)$$

where X is the typical elongation of a drop, $\gamma = \nu/\rho d^2$ is the attenuation constant of vibration (ν is the viscosity)⁹, and

$$\omega \approx 2\pi(\sigma/\rho V)^{1/2}$$

$V = d^3/2$ is the drop volume in the equilibrium position.

The average degree of elongation e at some time t can be determined from X^2 by averaging over the random phase, δ

$$e^2 = \langle X^2 \rangle_\delta = \frac{X_0^2}{2} \exp(-2\gamma t)$$

Because of the exponential dependence of viscosity on temperature, it is assumed that at a certain moment $t = t_s$ the particle solidifies and 'memorises' its shape.

It can be shown easily that $t_s \approx d$ and thus $\gamma \approx d^{-2}$; then

$$e = \frac{X_0}{\sqrt{2}} \exp\left(-\frac{\text{const.}}{d}\right)$$

Thus, the average elongation of particles, e , markedly decreases with decreasing particle size; just this has been observed in lunar particles³.

The popular theory that rotating drops should form elongated and dumb-bell¹⁰⁻¹² shaped particles is not valid. Rotating particles will sometimes acquire an S shape.

Let τ be the time during which a spherical particle is elongated to the length $2l$ with mean velocity $v = l/\tau$. If ω is the angular velocity of particle rotation then $a_\perp = 2\omega v$ is the Coriolis acceleration, and $S = a_\perp \tau^2/2$ is the transverse deformation. On the other hand, the longitudinal acceleration is $a_\parallel \approx \omega^2 l$ and $l \approx a_\parallel \tau^2/2$, hence one gets the value of $S/l = \omega \tau \approx \sqrt{2}$. Since $S/l > 1$, bending of the rotating particle is quite significant. Rotation about a dumb-bell symmetry axis also does not bring about the dumb-bell shape because of the instability of forms that are thus generated².

Thus, the variation principle for determining static equilibrium shapes^{10,11} cannot be used. A great number of elongated particles could be generated as a result of vibrations, but only a

few of the crescent particles observed could be formed by rotation.

Pools of melted magma

We can also show that no 'pools' or 'lakes' of melted magma are formed by meteorite impact on the crater floor, which could have been the source of large particle formation. The velocity u of the medium dragged behind the shock wave is related to the specific energy by $\varepsilon \approx u^2/2$; therefore $u \approx (2\varepsilon_m)^{1/2}$ where ε_m is the specific melting heat $\sim 5 \times 10^9$ erg g⁻¹ (ref. 7) with $u \gtrsim 10^5$ cm s⁻¹. It is clear that most material will be ejected a long way except for some from the region near the crater wall. The formation of a boundary layer can be easily shown to occur by making an estimate of Reynolds number for the given outflow ($Re > 10^3$) for craters with $D_s > 1$ cm. If we estimate the boundary layer thickness, δ_0 , in a usual way, then $\delta_0 \sim 10^{-2} \sqrt{D_s}$ will be obtained where D_s (cm) is the crater diameter. Values $\delta_s = 1$ cm and 0.03 cm are obtained for craters with $D_s = 10^2$ m and 0.1 m respectively.

It is clear that the cooling layer $\delta_0 \sim 0.03$ cm thick will not flow down over the rough soil, and the layer $\delta_0 = 1$ cm for a 100-m crater will give the maximum depth for a pool ~ 30 cm. These figures are gross overestimates, since the edge of the boundary layer has a higher velocity than the bulk of the material, which will join the disintegrated soil.

Thus, only vitrified surfaces are formed in meteorite impact, not 'pools' and 'lakes'. Therefore, the large particles of the right peak in the size distribution also cannot be formed. They must have been formed in endogenic volcanic processes.

Secondary particles

These very simple estimates show that new regularly shaped particles are not formed during the collision of secondary particles (meteorite interaction products) with the lunar surface. Indeed, such particles have a velocity, v , lower than the escape velocity ($v_e \approx 2.4$ km s⁻¹). The corresponding energy, ε , in the shock wave is $\varepsilon \approx v^2/2 \approx 5 \times 10^9$ erg g⁻¹ which lies on the boundary of specific melting heat. As is well known⁷, an approximately tenfold energy storage is required for starting the spraying process, and melting is never experimentally observed for such velocities.

A simple model

We may construct an elementary model of the evolution of lunar surface material where a depth h is uniformly affected by systematic meteorite bombardment. The mass fraction of primary material is designated by μ_1 , crushed material by μ_2

overmelted material by μ_3 and crushed glass by μ_4 . Then they follow the following set of equations

$$\frac{d\mu_1}{d\tau} = -(\alpha + \zeta)\mu_1; \quad \frac{d\mu_2}{d\tau} = \zeta\mu_1 - \alpha\mu_2$$

$$\frac{d\mu_3}{d\tau} = \beta\mu_4 - \alpha\mu_3; \quad \frac{d\mu_4}{d\tau} = \alpha(\mu_1 + \mu_2 + \mu_3) - \beta\mu_4$$

where α , β , ζ are the relative volumes of 'pit', 'halo' and 'crushing zone', respectively. From ref. 2, we obtain $\alpha \approx 10^{-2}$; $\zeta = 1 - \alpha$; $\beta \approx \zeta \approx 1$, and τ is dimensionless geological time.

Assuming, for simplification, that $\beta = \zeta = 1 - \alpha$ and choosing the initial conditions $\mu_2(0) = \mu_3(0) = \mu_4(0) = 0$ and $\mu_1(0) = 1$ the solutions of the equations are

$$\mu_1(\tau) = e^{-\tau}; \quad \mu_2(\tau) = e^{-\alpha\tau}(1 - e^{-\beta\tau})$$

$$\mu_3(\tau) = \beta - e^{-\alpha\tau} + \alpha e^{-\tau}; \quad \mu_4(\tau) = \alpha(1 - e^{-\tau})$$

From these solutions it follows that the events develop with two time constants $\alpha^{-1} \gg 1$ and $\beta^{-1} \approx 1$. Primary material (μ_1) is rapidly (within time ≈ 1) changed to crushed primary material (μ_2), which grows up to $\tau \sim 1$, then slowly ($\sim \alpha^{-1}$) decreases because of the remelting of glass of regular shape (μ_3). These particles produce the crushed glass on bombardment, and its fraction μ_1 rapidly grows. With $\tau \gg \alpha^{-1}$ all the material is transformed into glass of regular (μ_3) and irregular (μ_4) shape, and $\mu_3 \rightarrow \alpha$, $\mu_4 \rightarrow 1 - \alpha$.

Ratios of $\mu_1 : \mu_2 : \mu_3 : \mu_4$ are additional experimental chronological characteristics for lunar surface sites.

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Volatilisation of elements from melts of lunar material

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We discuss here the theory of the volatilisation of components of molten rocks and develop a model for this process, which enables us to draw some conclusions about such phenomena on the Moon.

CHEMICAL analyses of the surface of the Moon have shown that it is depleted in such volatile elements as Na and K when compared with similar terrestrial rocks. To explain this, two contradictory hypotheses¹ have been put forward: first, that the

magmatic material is depleted in these elements because of volatilisation from magmatic melts on the surface of the Moon and second, that magmatic melts effused on the surface were primordially depleted in such elements. To elucidate this problem, numerous attempts have been undertaken to simulate the depletion process by heating melts of terrestrial rocks to high temperatures, both in a vacuum and in an atmosphere of inert gases²⁻⁷. There has been no adequate physical model, however, and it has been impossible to extend the experimental results to lunar conditions.

Here we develop a theory of volatilisation and show that the

presence of oxygen has a decisive influence on the process kinetics, especially on the final state of the melts. This provides the basis for the laboratory simulation of volatilisation and also for an understanding of lunar processes.

Basic assumptions

Thermodynamically, a high-temperature melt placed in a vacuum chamber with 'cool' walls is a significantly non-equilibrium 'open' system. In such a system, the evaporation of a volatile component should proceed until it is fully evaporated. Yet, experiments have revealed the existence of plateaux in component concentrations which are stable even after long exposure. Moreover, the heights of the plateaux were found⁶ to depend on the pressure in the experimental vacuum chamber, but since in the pressure range involved ($p < 10^{-3}$ mmHg) the return coefficient Z is $\ll 1$, these plateaux cannot be caused by the pressure-dependent return of volatile molecules to the melt.

The only possibility for explaining the data is that molecules of the volatile elements reaching the surface may either leave the melt or enter a non-volatile state through a chemical reaction. Oxidation is just such a reaction, the oxygen being adsorbed on to the melt surface from residual gas in the unit.

The model

For the mathematical investigation of the volatilisation process, the melt can be assumed, with reasonable accuracy, to be a regular solution⁸ of dissolved Na (hereafter by Na we mean any volatile element). Denoting the volume concentrations (cm^{-3}) of Na in the melt by x_1 in the volatile and x_2 in the non-volatile states, one can deduce the following kinetic equations for the concentrations

$$\frac{dx_1}{dt} = k_{21}x_2 - k_{12}x_1 - j \exp(-\beta x_1)x_1 \quad (1)$$

$$\frac{dx_2}{dt} = k_{12}x_1 - k_{21}x_2 \quad (2)$$

where k_{12} and k_{21} are the rate constants for transition from state 1 to 2 and from 2 to 1, respectively. The quantity j characterising the rate of evaporation is

$$j = \frac{(1-Z)\alpha_v \mu}{h\rho(2\pi mkT)^{1/2}} \exp(A/RT)p_1(T) \quad (3)$$

In this case $p_1(T)$ is the vapour pressure for pure Na at temperature T , R the universal gas constant, μ and ρ are the molecular weight and density of the solvent, A the heat of solution of Na in the melt per unit of concentration, k the Boltzmann constant, m the mass of the Na atom, Z and α_v are the return and evaporation coefficients and h is the melt depth.

The value h is taken to be sufficiently small for the time of transport of the Na molecule to the surface, compared with the

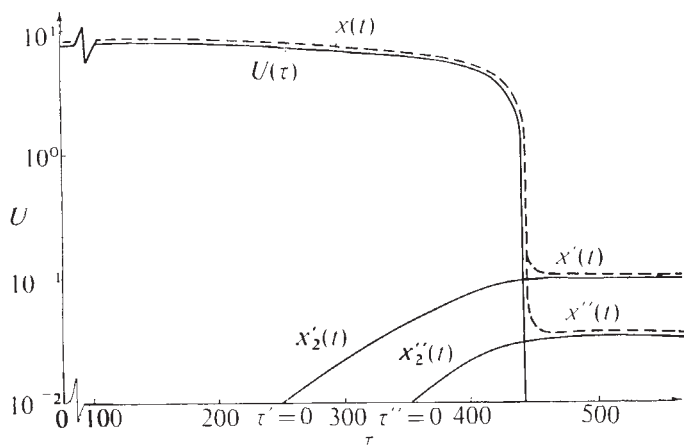


Fig. 1 $U(\tau)$, universal dependence, and $x'(t) = x_1(t) + x_2(t)$ and $x''(t) = x_1(t) + x_2''(t)$ dependences.

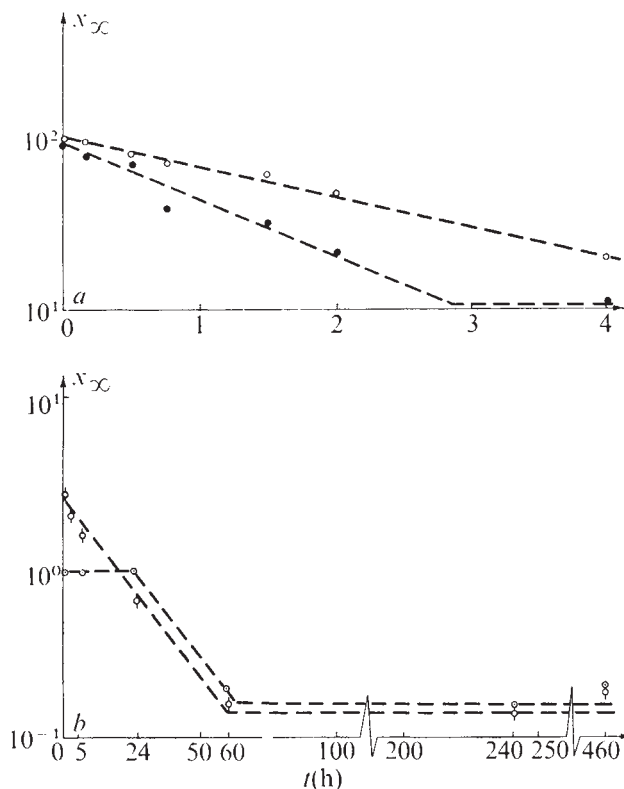


Fig. 2 Volatility kinetics for Na and K from the melt of the terrestrial rock in a vacuum, a, $T \approx 1,050^\circ\text{C}$; $p \approx 10^{-6}$ mmHg (ref. 7). b, $T \approx 1,150-1,175^\circ\text{C}$; $p \approx 10^{-4}-10^{-5}$ mmHg (ref. 2); open circles with and without dot, K; closed circles and open circles with ticks, Na.

other characteristic times of the process, to be neglected. The exponent factor in equation (1) is

$$\beta = \frac{2A\mu}{\rho RT} \quad (4)$$

Solutions

An analysis of the solutions of equations (1) and (2) for $t \rightarrow \infty$ and of the experimental data shows that the inversion from the non-volatile state 2 to the volatile state 1 is practically absent for

$$k_{21} \ll j + k_{12}; \quad k_{21} \ll j; \quad k_{21} \ll k_{12}$$

After neglecting the terms proportional to k_{21} in equations (1) and (2) the system is solved in quadratures

$$\int_{x_{10}}^{x_1(t)} \frac{dx}{x[\exp(-\beta x) + (k_{12}/j)]} = -jt \quad (5)$$

$$x_2(t) = x_{20} + k_{12} \int_0^t x_1(t') dt' \quad (6)$$

In equations (5) and (6) we have labelled the quantities corresponding to the initial state by 0. For the qualitative analysis of the solutions (5) and (6), we neglect the term k_{12}/j in (5) and find

$$E_1^*(\beta x_1) = E_1^*(\beta x_{10}) - jt \quad (7)$$

where $E_1^*(z)$ is the integral exponent determined and tabulated in ref. 9.

Analysis

Formula (7) gives the universal curve of the dimensionless concentration as a function of dimensionless time, $\tau = jt$.

To the various initial concentrations $U_0 = \beta x_{10}$ correspond shifts of the reference of time to the universal curve (7) by the value $E_1^*(U_0)$. Figure 1 shows that the total concentration $x(t) = x_1(t) + x_2(t)$ has two types of kinetic dependence: a two-step and a one-step curve. The first is realised if $U_0 \equiv 2A\mu x_{10}/\rho RT \gtrsim 5$ while the second is realised when $U_0 < 5$. In particular, if $U_0 < 1$ then $x(t)$ tends to drop immediately after the period of exponential decrease of $x_1(t)$. These types of behaviour have been observed experimentally^{2,3,6,7} (Fig. 2).

For investigating the pressure and time dependences we consider the case $U_0 < 1$ which was involved in these experiments. In this case $\exp(\beta x) \approx 1$ and the equations (5) and (6) yield for $t \rightarrow \infty$

$$x_\infty = x_{20} + x_{10}[k_{12}/(j + k_{12})]; \quad x_\infty \equiv [x_1(t) + x_2(t)]_{t \rightarrow \infty} \quad (8)$$

The two terms of equation (8) with $x_{20} \neq 0$ cannot give the observed power-type pressure dependence x_∞ (ref. 6 and Fig. 3). Therefore it is natural to put $x_{20} = 0$ in equation (8).

It can be shown that in the high vacuum region the pressure dependence of j is unimportant, therefore the dependence of x_∞ on p is determined by the value $k_{12}(p)$. Here the experiment gives $k_{12} \propto p^\gamma$, as in the absorption isotherm of Freundlich¹⁰ for the concentration $n(p)$ of the molecules (atoms) adsorbed on the surface from the residual atmosphere.

It thus appears that the only possible explanation of the presence of the power-type pressure dependence is the assumption that Na is oxidised on the melt surface by oxygen sorbed on the surface. In this case

$$k_{12} = ap^\gamma \exp\left(-\frac{Q - q_v}{RT}\right) \propto n(p) \exp\left(-\frac{Q}{RT}\right) \quad (9)$$

Fig. 3 a, b, Volatility of Na and K from the melt of aluminiferous and tholeiitic basalts in vacuum ($T = 1,300$ and $1,500^\circ\text{C}$) (ref. 6).

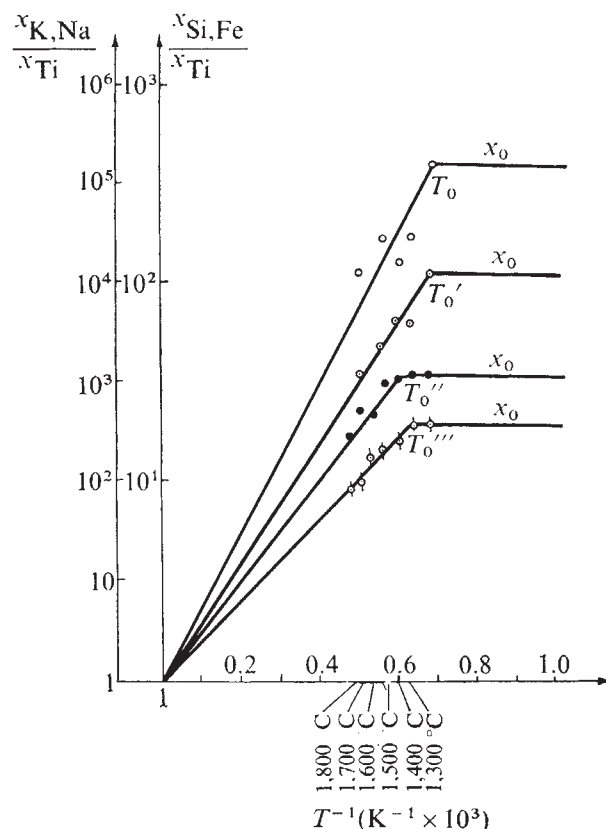
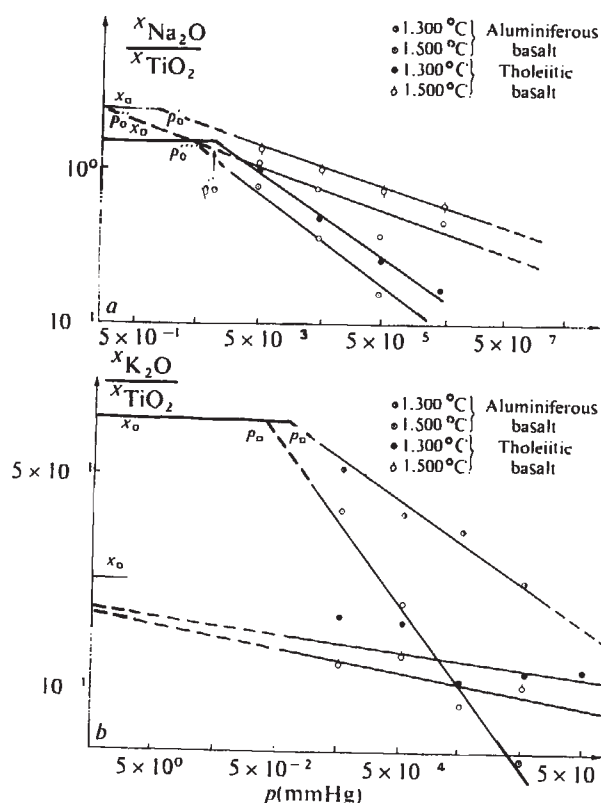


Fig. 4 Summary plot of the volatility dependence of Na, K, Si and Fe in vacuum ($p < 10^{-6}$ mmHg) from the melt of basalt^{6,6}. A heat of reaction, ψ , is determined by the bend angle at the $x_\infty(T)$ curve $\psi_K = 31.2$ kcalorie mol⁻¹; $\psi_{Na} = 21.6$ kcalorie mol⁻¹; $\psi_{Si} = 5.14$ kcalorie mol⁻¹; $\psi_{Fe} = 5.0$ kcalorie mol⁻¹. $\circ$, K/Ti; $\odot$, Na/Ti; $\bullet$, Si/Ti; $\ominus$, Fe/Ti.

where a is a constant, q_v is the heat of adsorption (several units of kcalorie mol⁻¹) and Q is the energy of oxidation activation (of the same order as q_v (ref. 10)).

Let us factorise the main temperature dependence in equation (3)

$$j = b \exp\left(-\frac{L - A}{RT}\right); \quad b = b(T) \approx \text{const.} \quad (10)$$

where L is the heat of evaporation of pure Na (≈ 25 kcalorie mol⁻¹). Now we can write for x_∞ the final expression

$$x_\infty = x_{10} \frac{k_{12}}{j + k_{12}} = x_{10} \frac{ap^\gamma \exp(-(Q - q_v)/RT)}{b \exp(-(L - A)/RT) + ap^\gamma \exp(-(Q - q_v)/RT)} \quad (11)$$

From equation (11) it follows that there exist two pressure and temperature regions ($j \gg k_{12}$ and $j \ll k_{12}$) which account for the presence of specific kinks p_0 and T_0 on the curves $x_\infty(p)$ and $x_\infty(T)$ (Figs 3 and 4). Because $(L - A)$ is always $> (Q - q_v)$ in the low temperature region $T < T_0$, $k_{12} \gg j$ and $x_\infty \approx x_{10}$, and practically no volatilisation occurs. In the region $T > T_0$, $j \gg k_{12}$ and volatilisation occurs rapidly, in which case

$$x_\infty = x_{10}(k_{12}/j) = x_{10}(a/b)p^\gamma \exp(-(Q - q_v + A - L)/RT)$$

which demonstrates the power-type pressure and exponential-type temperature dependence.

At reasonably high pressures $p \gg p_0$, $j \ll k_{12}$, volatilisation is almost complete and the pressure dependence disappears⁶.

Lunar applications

Thus, the picture presented here facilitates explanation of all the peculiarities of the experimentally observed dependences of x_∞ on t , p and T in the region of low pressures and moderate temperatures. As regards volatilisation on the Moon, our theory is applicable when we may neglect transport processes, that is, in a fairly thin layer. In this case the estimate of U_0 using the parameters of the lunar material³ indicates that only for $x_0 \leq 5\%$ could the exponential volatilisation regime $x_1(t) \approx x_{10} \exp(-jt)$ have occurred.

Heating lunar material in a laboratory vacuum demonstrates that it is in the exponential kinetic volatilisation regime, and therefore that the process occurred on the Moon at $T > T_0$, which could have led to the complete disappearance of Na from the surface material if rather rapid cooling had not taken place. The observed low concentrations of free Na in lunar material³ also point to the almost complete absence of oxygen in the ancient Moon's atmosphere. The emergence of the curves $x(t)$ on to the plateau obtained in a number of laboratory experiments^{2,3,6,7} is a purely instrumental effect associated with the presence of oxygen in the residual atmosphere of experimental equipment. We note that the process considered here gives a possible mechanism for the 'disappearance' of oxygen and other active gases from the atmosphere of the Moon and planets: sorption on the melted material on the surface, with subsequent chemical bonding. For determining

the partial pressure of oxygen in the Moon's atmosphere at the instant of cooling of the lunar material down to $T \lesssim T_0$, it is necessary to perform an investigation of volatilisation from lunar rocks in a vacuum-pumping assembly with $p \approx 10^{-12}$ – 10^{-13} mmHg. If a plateau is revealed on the curve $x(t)$, then its height will define p_{O_2} in the Moon's atmosphere according to our theory. The suggested theory has been formulated for isothermal volatilisation and applies therefore primarily to exogenic (volcanic) processes accompanied by the formation of large accumulations of melted material. The common viewpoint, attributing volatilisation to meteorite collisions, is wrong, since they do not produce significant volatilisation of Na, at least in single collisions (see ref. 11). This results from the fact that in collisions relatively small^{11,12} accumulations of liquids are formed, which solidify before significant volatilisation occurs.

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Partial sequences of 16S rRNA and the phylogeny of blue-green algae and chloroplasts

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Partial sequence analyses of 16S ribosomal RNAs of blue-green algae and chloroplasts reveal that blue-green algae are typically prokaryotic and related to the bacilli, that red algal chloroplasts are probably of blue-green algal origin, and that euglenoid and red algal chloroplasts may have arisen independently.

WE have begun to assemble sequence information on the 16S rRNAs of the blue-green algae and on the evolutionary homologues of these molecules in eukaryotic algae: chloroplast 16S and cytoplasmic 18S rRNAs. The assembled data should serve to determine the relatedness of blue-greens to bacteria, to establish taxonomic structure within the blue-greens, and to determine phylogenetic relationships between blue-green algae and chloroplasts.

Blue-green algae are a major group of photosynthetic prokaryotes readily distinguishable from photosynthetic bacteria by their possession of oxygen-evolving photosynthetic mechanisms otherwise found only in eukaryotic algae and higher plants. There is little quantitative information on which to base measurements of the extent to which this group is related to any of the major groups of bacteria, either photosynthetic or non-photosynthetic. Woese and his collaborators^{1–3} have shown the use of 16S rRNA sequence information (in the form of 'T₁ oligonucleotide catalogues') in establishing phylogenetic relationships among bacteria. Acquisition of similar information for blue-greens should

allow their unambiguous positioning among the prokaryotes.

Classical taxonomies of blue-green algae rest on morphological characters perhaps suitable for the structurally differentiated multicellular filamentous forms but wholly inadequate for classification of unicellular species. The latter are likely to be of greater antiquity⁴ and phylogenetic diversity, showing a range of DNA GC contents (35–71 mol %) nearly as large as that characterising the most distantly related bacteria (25–75 mol %). Quantitative treatments such as those now being developed by Stanier⁴ and that which is possible for RNA sequence data are required for the construction of a rational taxonomy for this group.

Recognition of the near identity of photosynthetic mechanisms in blue-greens and eukaryotic photosynthesizers underlies the now generally accepted notion that chloroplasts, the semi-autonomous, self-replicating organelles of eukaryotic photosynthesis, evolved from oxygen-producing photosynthetic prokaryotes resembling blue-green algae^{5–10}. Two specific hypotheses concerning this evolution can currently be entertained: (1) the "endosymbiont hypothesis"^{6,7}, in which chloroplasts are assumed to descend from photosynthetic prokaryotes living as endosymbionts within the cytoplasm of heterotrophic protoeukaryotic cells to which they were phylogenetically unrelated, and (2) the "direct filiation" hypothesis^{8–10}, in which compartmentation of a single photosynthetic cell is assumed to have given rise to the separate and functionally distinct DNA-containing organelles now recognised as nuclei and chloroplasts. With

Oligonucleotide catalogues

The requisite sequence information can be obtained with techniques developed for bacterial 16S rRNAs^{1-3,11-14}. In brief, these involve preparation of high specific activity ³²P-labelled 16S (or 18S) rRNA, its complete digestion with ribonuclease T₁ to yield approximately 500 Gp terminated oligonucleotide fragments ranging in length from one to about 15 nucleotide residues, resolution of these fragments by two-dimensional electrophoresis, and determination of the sequence of each oligonucleotide by appropriate secondary and tertiary treatment with nucleases of different specificity. This generates for each rRNA a unique catalogue of oligonucleotides. Enumeration of oligonucleotides of identical (coincident) sequence in catalogues for rRNAs from two different organisms or organelles yields a measure of their relatedness. Some such sequence coincidences of course arise by chance, and the majority of coincidences between dimers, trimers and tetramers will in fact be of this sort. For two completely unrelated (random sequence) RNAs of the size and composition of prokaryotic 16S rRNAs there should, however, arise by chance only about 15 coincidences among pentamers, only about 2 coincidences among hexamers, and, on average, no coincidences among oligonucleotides of greater length^{3,15}. Even the most distantly related 16S rRNAs considered here show at least twice this level of sequence similarity and (when oligonucleotides of less than five residues are excluded from the analysis) conclusions of considerable statistical significance can be drawn. For instance, the probability that chance alone accounts for the level of oligonucleotide sequence coincidence observed between the 16S rRNAs of *Synechococcus* 6301 and *Porphyridium* chloroplasts (see below and ref. 15) is approximately 10⁻³¹. Table 1 displays recently completed catalogues for the three unicellular blue-green algae described by Stanier⁴ as strains 6714, 6701 and 6308 (the last being the Wellesley strain of *Gloeocapsa alpicola*). All three are of what he, on the basis of morphological, physiological and biochemical characters, designates typological group IIA (*Aphanocapsa*)⁴. The GC contents of their DNAs are respectively 47.4, 35.7 and 34.7 mol%. Table 1 also indicates, for each sequence present in one or more of these three strains, its presence or absence in published catalogues of the 16S rRNAs of strain 6301 (ref. 16) (formerly *Anacystis nidulans*), a unicellular blue-green alga of typological group IA (*Synechococcus*) with a DNA GC content of 55.1 mol% (ref. 4); the chloroplasts of a species of *Porphyridium*¹⁵, a unicellular (eukaryotic) marine red alga; and the chloroplasts of *Euglena gracilis*, as recently described by Zablen *et al.*¹⁷.

We can derive from complete data matrices (Table 1) much useful information which will be lost when such data are further reduced to generate measures of similarity (see below). The following are a few of the kinds of comparison which can be made.

(1) There are 39 oligonucleotides (pentamers and larger) which are present in all four blue-green algal strains, and which we here temporarily designate "blue-green invariants". (The number of invariants will certainly fall as more catalogues become available.) Of the 39, 22 are identical to oligonucleotides identified by Woese *et al.*¹ as "universal" among bacterial 16S rRNAs (present in at least 24 of 27 species catalogued), and a further 6 have been described by them as "conserved" (present in at least 14 of 27). Of the 11 blue-green invariants which are neither universal nor conserved in bacteria, two (AUACCCUG and UACUACAAUG) are readily identified as differing by only a single residue from such sequences (AUACCCUG and CUACAAUG). Conversely, of the 26 oligonucleotides (pentamers and larger) described by Woese *et al.*¹ as universal to bacteria, 22 are blue-green invariants, two (the above-mentioned) differ by a single residue from invariants,

and one (CAACUCG) is found unaltered in 6301 and 6714 but altered (AAACUCG) in 6701 and 6308 (and both chloroplasts). Only one (AAUACG) cannot be readily identified in some form in each of the four blue-green species. Thus, most if not all sequences identified (by virtue of their conservatism) as essential to functions of bacterial ribosomes probably retain similar functions in the ribosomes of blue-green algae.

(2) *Porphyridium* chloroplast 16S rRNA contains 64 oligonucleotides (including 32 invariants) which are found in one or more blue-green algal 16S species, while *Euglena* chloroplast 16S contains only 45 such oligonucleotides (including 25 invariants). Twenty-eight of the oligonucleotide sequences shared by one or more blue-green rRNAs and *Porphyridium* chloroplast 16S are found only in this chloroplast rRNA (not also in that of *Euglena*). By contrast, only nine of the sequences shared by blue-green rRNAs and *Euglena* chloroplast 16S are found uniquely in this species (not also in *Porphyridium* chloroplast 16S). Thus the 16S rRNA of *Porphyridium* chloroplasts shows substantially more homology with blue-green algal rRNAs than does the 16S rRNA of *Euglena* chloroplasts (see also below). This was not apparent when the only blue-green algal catalogue available was that of strain 6301 (refs 15 and 17).

(3) Each of the four blue-green 16S rRNAs contains a different variant of the dodecamer UUACXYZAACCG, the variable portion XYZ being CCU for 6301, UCU for 6714, CUU for 6701 and CUC for 6308. It is possible to visualise each sequence as a hairpin with two or three of the variable nucleotides unpaired in the loop and the stem having in three cases four and in one case (6308) three base pairs (including for this purpose one base pair involving the G located 5' to these sequences in intact 16S rRNA). The 6714 variant of this dodecamer is found also in *Porphyridium* chloroplast 16S rRNA, but no variant of it is detectable in *Euglena* chloroplast 16S rRNA, nor in any of the bacterial rRNAs described by Woese and his coworkers. We know of no other instance in which such a highly variable region has been found embedded within an otherwise very conservative 16S rRNA oligonucleotide.

Analysis and implications

For taxonomic purposes, the data of Table 1 must be reduced to yield similarity coefficients S_{ij} which measure relatedness in all pairwise comparisons between any two RNAs i and j . One method for doing this is described in the legend to Table 2, and values of S_{ij} computed in this way are shown above the diagonal in that table. Data used include not only the four blue-green algal and two chloroplast 16S rRNA catalogues but, in addition, catalogues obtained by Woese and coworkers for three rather diverse bacterial species. It is possible to derive for each S_{ij} a new value, C_{ij} , which can be interpreted as the probability of any single nucleotide residue having remained unchanged during the evolutionary divergence of rRNAs i and j , or, alternatively, as the percentage sequence homology between these RNAs. Values of C_{ij} (as a percentage) are shown below the diagonal in Table 2.

From Table 2, we see that prokaryotic and chloroplast 16S rRNAs are highly conserved molecules; no pair of them shows less than 80% homology. By contrast, the single cytoplasmic 18S rRNA for which we have appropriate data (that of *Porphyridium*) shows no detectable homology with prokaryotic or chloroplast 16S rRNAs (ref. 15). It shares with *Porphyridium* chloroplast 16S, for instance, no oligonucleotides of more than six residues, and no more than the number of coincident pentamers and hexamers expected to arise by chance. (It should be recognised, however, that any pair of molecules for which C_{ij} is less than about 60% will not show detectable homology by this method.)

Much of the conservatism observed among prokaryotic

and chloroplast 16S rRNAs is due to the retention by most of them of sequences designated by Woese *et al.*¹ as universal or conserved among bacteria. There are in fact 18 sequences which are common to all nine 16S species discussed here, and any two RNAs sharing only these 18 sequences would give a value of C_{ij} of about 68%. As a consequence of 16S conservatism, values of C_{ij} vary over a restricted range, and taxonomic inferences must be drawn

Table 2 Relatedness in pairwise comparisons

	Similarity coefficient, S_{ij}								
	<i>E. coli</i>	<i>B. subtilis</i>	<i>R. spheroides</i>	6301	6714	6701	6308	<i>Porphyridium</i>	<i>Euglena</i>
<i>E. coli</i>	33	37	31	31	28	28	27	27	
<i>B. subtilis</i>	86	35	37	34	32	32	34	26	
<i>R. spheroides</i>	88	87	32	33	30	29	30	23	
6301	85	88	86	49	44	41	36	35	
6714	85	87	86	92	58	52	47	32	
6701	84	86	85	90	94	83	43	33	
6308	84	86	84	89	92	98	42	32	
<i>Porphyridium</i>	83	87	85	88	91	90	89	36	
<i>Euglena</i>	83	82	80	87	86	86	86	87	

% Homology, C_{ij}

There are several ways in which the data of Table 1 can be used to derive single values (similarity coefficients S_{ij}) which measure the relatedness of any pair of RNAs i and j . That which seems most reasonable to us defines S_{ij} as

$$\left[200 \sum_{N=5}^{\infty} k_{N,ij} N \right] / \sum_{N=5}^{\infty} (t_{Ni} + t_{Nj}) N$$

where N is oligonucleotide length (in residues), $k_{N,ij}$ is the number of N -mers common to the two RNAs, and t_{Ni} and t_{Nj} are the total number of N -mers obtained from RNAs i and j , respectively. S_{ij} is thus equivalent to 200 times the number of individual nucleotide residues present in sequences (pentamers and larger) which are common to two rRNAs divided by the number of individual residues present in all sequences (pentamers and larger) found in both rRNAs. It will range in value from 100 for identical molecules down to 10–15 for totally unrelated species. (Chance sequence coincidences prevent S_{ij} from reaching zero in such cases.) Table 2 shows (above the diagonal) values of S_{ij} computed for the 36 possible pairwise comparisons involving the blue-green and chloroplast catalogues and, in addition, representative bacterial 16S rRNA catalogues obtained by Woese and his collaborators for *Escherichia coli*^{13,14}, *Bacillus subtilis*¹⁹, and *Rhodospseudomonas spheroides*²⁰ (a photosynthetic bacterium). For certain purposes it is useful to convert values of S_{ij} to values (C_{ij}) for the probability of any single nucleotide residue having remained unchanged during the evolutionary divergence of RNAs i and j , since the latter has more obvious physical significance (and can be equated with “% homology”). Such conversion was made with reference to a standard curve whose construction involved the following considerations. The nine rRNAs for which data are presented in Table 2 have nearly the same G content and hence approximately the same number of oligonucleotides of any length N . We define t_N as the average for the nine RNAs of the total number of N -mers and require that two hypothetical RNAs i and j each have exactly t_N N -mers. If C_{ij} is the probability of conservation of any single nucleotide during the evolutionary divergence of RNAs i and j , then the likelihood that a given N -mer will similarly be conserved is C_{ij}^{N+1} (the probability that all N residues as well as the preceding G have remained unchanged). Of t_N N -mers originally present, one thus expects on average $t_N C_{ij}^{N+1}$ to have been conserved and $t_N(1-C_{ij}^{N+1})$ to have changed. Of the $t_N(1-C_{ij}^{N+1})$ “new” N -mers which replace them, an average of $t_N(1-C_{ij}^{N+1})^2/3^{N-1}$ will by chance have the same sequence as one of the original N -mers which was lost¹⁴. Thus the total number of coincidences observed among N -mers in RNAs i and j will on average be

$$k_{N,ij} = t_N [C_{ij}^{N+1} + t_N(1-C_{ij}^{N+1})^2/3^{N-1}]$$

Using values of $k_{N,ij}$ calculated for different values of C_{ij} , a curve relating C_{ij} to S_{ij} for hypothetical RNAs of the sort described was constructed. Values of C_{ij} corresponding to values of S_{ij} shown above the diagonal in Table 2 were read from this curve and are shown (as % homology) below the diagonal.

on the basis of small differences between them. Such small differences are significant. For instance, that between 88 and 90% corresponds to a 17% difference in S_{ij} , or to one additional oligonucleotide sequence coincidence for each of the oligonucleotide size classes $N=5$ through $N=10$. Taxonomic distinctions of the type discussed here may, however, perhaps be more readily recognised in comparing values of S_{ij} .

The blue-green algae taken together are not demonstrably more distant from certain bacterial groups (as represented by *Escherichia coli*, *Bacillus subtilis* and *Rhodospseudomonas spheroides*) than some of these groups are from each other. For instance, they show on average as much homology (86.8%) with *B. subtilis* as the latter shows with *E. coli* (86%). Although the range of values for S_{ij} and C_{ij} in all pairwise comparisons between individual blue-greens and bacteria is small (28–37 and 84–88%, respectively), each of the four blue-green 16S rRNAs is more closely related to *B. subtilis* 16S than it is to *R. spheroides* 16S, and more closely related to the latter than it is to *E. coli* 16S rRNA. The consistency of this pattern supports an earlier suggestion (based only on data for 6301) that blue-greens are more closely related to the bacilli than to the enterics¹⁶. This relationship extends to post-transcriptional modification patterns; modified oligonucleotides are identical in all four blue-greens and more similar to those of the bacilli than to those of the enterics.

As expected, each of the four blue-green 16S rRNAs is more similar to every other blue-green 16S than it is to any of its bacterial homologues. Strains 6701 and 6308 show most similarity, their homology being as high as that characterising members of the same genus in many bacterial groups (C. R. Woese, personal communication). These two are in turn more closely related to strain 6714 than any of these three is to strain 6301. These findings are in full accord with Stanier's⁴ classification of 6701, 6308 and 6714 as members of a typological group (IIA) separate from that containing 6301 (IA).

Porphyridium chloroplast 16S rRNA is unquestionably prokaryotic in nature. Data on this molecule were first presented¹⁵ when only one blue-green algal 16S rRNA (that of 6301)¹⁶ had been catalogued. We were at that time puzzled that homology between the two (88%) was not substantially greater than that between chloroplast 16S rRNA and the 16S of a bacterium (*B. subtilis* (87%)), and expressed the hope that other blue-green algal 16S rRNAs would show greater specific homology with the chloroplast molecule. Indeed, each of the newly catalogued type IIA blue-green algal 16S rRNAs shows specific high homology with chloroplast 16S. Values of S_{ij} or C_{ij} for the three relevant pairwise comparisons are substantially greater than that for any other pairwise comparison involving *Porphyridium* chloroplast 16S (including that with *Euglena* chloroplast 16S). In fact, these values are on average (average $S_{ij}=44$, $C_{ij}=90.0\%$) only slightly below those which characterise the relatedness of the three type IIA strains to 6301 (average $S_{ij}=45$, $C_{ij}=90.3\%$). Thus a specifically blue-green algal (rather than generally prokaryotic) ancestor for red algal chloroplasts can be inferred, and this ancestor is likely to have resembled a modern type IIA blue-green more closely than a modern blue-green of type IA.

Euglena chloroplast 16S rRNA is (as Zablen *et al.*¹⁷ have shown) also unquestionably prokaryotic, and manifests significantly more homology with each of the four blue-green algal 16S species than with any of the three bacterial rRNAs. The levels and patterns of these homologies are, however, altogether different from those which characterise *Porphyridium* chloroplast 16S. *Euglena* 16S rRNA shows most homology with that of strain 6301, and least with each of those of the three blue-greens of type IIA, the last values being significantly less than comparable values for *Porphyridium* 16S. The level of homology between the two

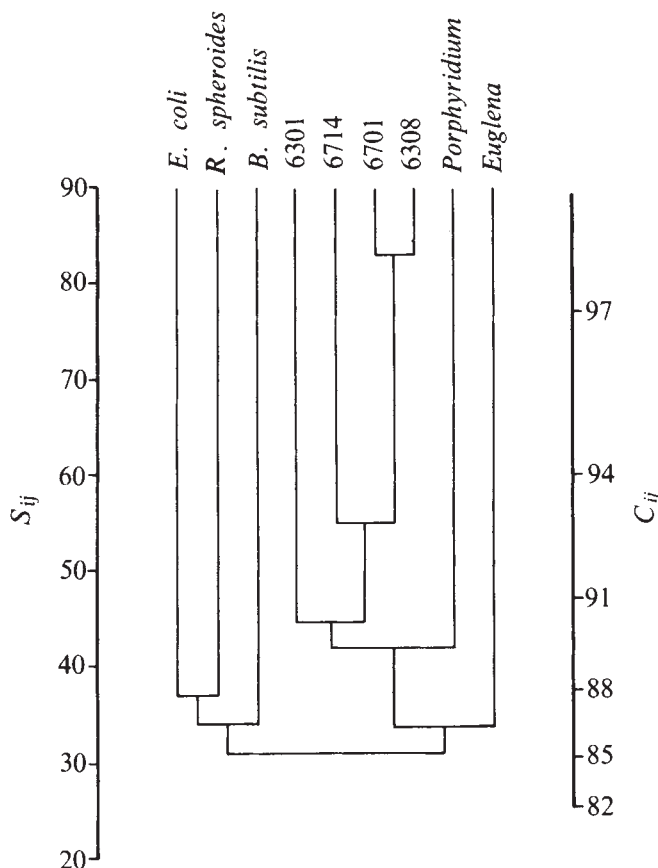


Fig. 1 A dendrogram summarising the relationships between nine prokaryotic and chloroplast 16S rRNAs.

chloroplasts themselves is less than any value of homology involving *Porphyridium* chloroplasts and any blue-green alga. If we take similarity coefficients as representative of evolutionary distance, then we must conclude that *Porphyridium* chloroplasts and blue-greens (at least those of type IIA) share a common ancestor more recent than any shared by *Porphyridium* chloroplasts and *Euglena* chloroplasts. Thus either type IIA blue-greens are "escaped chloroplasts", or, as seems more likely, chloroplasts of the red alga *Porphyridium* and those of the euglenoid *Euglena* arose independently from separate prokaryotic ancestors. Stanier^{4,6} has recently emphasised the fact that only the red algae (of all eukaryotic groups) contain chloroplasts which

are unarguably of blue-green algal origin. Other chloroplasts may derive from now extinct lines of oxygenic photosynthetic prokaryotes which, had they survived, might not be classified among the blue-green algae.

Figure 1 presents a dendrogram constructed from Table 2, using an unweighted pair-group method (with arithmetic averages)¹⁸. This dendrogram summarises the data presented here, and is not to be taken as a phylogenetic tree. We would be surprised, however, if the affinities which it indicates between prokaryotic and chloroplast 16S rRNAs were not reflections of true phylogenetic relationships between and among these organisms and organelles.

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Note added in proof: The preliminary catalogue for *B. subtilis* 16S rRNA used in the construction of Table 2 differs by a few oligonucleotides from the catalogue as it will be published¹⁹ (C. R. Woese, personal communication). New values of S_{ij} calculated with this final catalogue differ from those in Table 2 for the pairwise comparisons of *B. subtilis* with *R. spheroides*, 6714, 6701, 6308, *Porphyridium* chloroplast and *Euglena* chloroplast, and are respectively: 34, 35, 33, 33, 36 and 27. These minor changes are in no case of sufficient magnitude to alter significantly the conclusions drawn in the text.

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letters to nature

Are supernova explosions driven by magnetic springs?

SUPERNOVA explosions are generally believed¹ to be powered by the gravitational energy of a collapsing core of (Chandrasekhar) mass $\sim 1.4M_{\odot}$. There is a difficulty, however, with the radial momentum balance: how is the liberated energy converted into the radial motion of an ejected shell? Neither thermal pressure gradients nor neutrino pressures seem to be sufficient to accelerate matter to the observed several per cent of the velocity of light, even after the 1974 rise in the calculated

cross section for resonant neutrino scattering. On the other hand, there is increasing evidence that magnetic neutron stars^{2–3} form at the centre of (at least a large subclass of) supernovae. I should like to argue that their magnetic pressure is a serious candidate for driving the supernova motion.

According to stellar evolution theory^{4,5}, a sufficiently massive pre-supernova star is thought to develop a degenerate carbon-oxygen core of mass $\sim 1.4M_{\odot}$, radius $R_c \gtrsim 10^8$ cm, — matter density $\rho_c \lesssim 2 \times 10^{10}$ g cm⁻³ which becomes unstable to inverse β decay, and implodes to become a neutron star. The core essentially conserves⁶ its angular momentum after formation because molecular friction is negligible, and convective friction

is restricted to the shells between chemical discontinuity surfaces; from $\Omega\rho^{-2/3}=\text{const}$, and an initial angular velocity $\Omega_i \simeq 10^{-5} \text{ s}^{-1}$ we find $\Omega_c = \Omega_i(\rho_c/\rho_i)^{2/3} \simeq 10 \text{ s}^{-1}$. The fast spinning core is likely to be convective⁷, and to generate⁸ equipartition magnetic fields of strength $B_c = (4\pi\rho_c v_{\text{conv}}^2)^{1/2} \simeq 3 \times 10^9$ gauss. Magnetic flux conservation during core implosion results in neutron star magnetic fields $B_{\text{ns}} = B_c(\rho_{\text{ns}}/\rho_c)^{2/3} \simeq 4 \times 10^{12}$ gauss for $\rho_{\text{ns}} \simeq 4 \times 10^{14} \text{ g cm}^{-3}$, in agreement with observations of pulsars. On the other hand, backward extrapolation⁹ of pulsar angular frequencies Ω_p according to $\Omega_p \propto (t+t_1)^{-1/2}$ leads to $\Omega_p \simeq 4 \times 10^2 \text{ s}^{-1}$ 'at birth' (for $t_1 \sim 3 \times 10^2 \text{ yr}$) in the case of the Crab and the Vela pulsar, a value which is small compared with $\Omega_{\text{ns}} = \Omega_c(\rho_{\text{ns}}/\rho_c)^{2/3} \simeq 10^4 \text{ s}^{-1}$ for a new born neutron star, assuming conservation of angular momentum during core collapse. We infer that a new born neutron star loses angular momentum at a much higher rate than given by the power law above, thereby releasing its rotational energy $\sim MR^2\Omega^2/6 = 3 \times 10^{52} \text{ erg } \Omega_4^2$ on a short time scale ($\sim 1 \text{ min}$).

The detailed mechanism may be as follows: conservation of angular momentum during core implosion implies a ratio $E_{\text{rot}}/E_{\text{grav}} \sim R^3\Omega^2/2GM = \Omega_4^2 R_6^{-1}$,³ for $M=1.4M_\odot$, so that gravitational energy E_{grav} would be 100% converted into rotation for $\Omega_4=1$. Note that gravitational radiation cannot remove more than $(GM/Rc^2)^{3/2}(\Omega R/c)^4 \lesssim 10^{-3}$ of the rest energy Mc^2 , that is, it does not invalidate our estimate. For $\Omega_4 \simeq 1$, only a small fraction of E_{grav} would go into heat and oscillation. The fast rotating collapsed core will not swallow all the initial magnetic flux, and a winding of field lines is unavoidable in the highly conducting surrounding plasma. Magnetic stresses will soon force the plasma into corotation up to the velocity-of-light cylinder of radius $c/\Omega = 30 \text{ km } \Omega_4^{-1}$, and will drive matter towards relativistic speeds beyond it. Winding will increase the magnetic energy density—which temporarily lost significance during core implosion—roughly as $(\Omega/2\pi)^2$, and equipartition with rotational energy densities will be reached within $< 10^2 \Omega_4^{-2} \text{ s}$. In this way, the (huge) rotational energy of the imploded core is tapped by a magnetic spring; cf. ref. 10, where 30 times higher (than ours) initial magnetic field and an assumed axial symmetry led to a jet-like explosion along the rotation axis. Only $\sim 10^{-1} \Omega_4^2$ of this energy is needed to drive the supernova motion; the rest will heat the plasma by shear viscosity, and field line reconnection.

This generation of magnetic flux by differential rotation will operate only until some volume beyond the velocity-of-light radius has been evacuated, and the ordinary pulsar braking mechanism takes over (magnetic dipole radiation, an electromagnetically driven wind, or magnetospheric friction). This should happen on the same time scale as the build-up of the spring. The newly-born pulsar, having exerted a sizeable torque on the expanding shell, would be expected to have a strong toroidal magnetic field.

In conclusion, it is suggested that magnetic torques between an imploded magnetic core and a surrounding massive shell can explain both the radial momentum balance of supernova explosions, and the small 'observed' spin rates of pulsars at birth.

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Effects of back warming in cocoon stars

THERE now exists general agreement among astronomers that dust shells surrounding young stars are a relatively frequent occurrence, and attempts have been made to calculate some of the properties of such shells (see refs 1 and 2). One obvious possibility is that the dust absorbs the outgoing radiation from the star and re-emits it in the infrared, and this is perhaps the most acceptable explanation for a number of the infrared sources that have been detected in the Orion nebula and in other regions where star formation is suspected to be occurring. If the dust shell absorbs any radiation, then both its inner and outer surfaces will re-emit a certain proportion, though now at a different wavelength, so that some radiation will return to the central star—it is 'warming its own back' so to speak. It is interesting to ask how such a star will evolve when compared with the evolution of a normal pre-main-sequence star. A model for a contracting star which is receiving radiation from an external source has already been developed by the authors³ in connection with the evolution of Jupiter within the radiation field of the Sun, and here we apply this model to the situation just described.

It should be stressed that we are discussing the evolution of the central star only, the dust being regarded merely as a means of redirecting radiation back on to the surface of this star. We do not tackle the much more complex problem where the dust shell is itself modified because it has modified the star.

We shall also confine ourselves to a purely analytical discussion of the problem. We first need the Eddington⁴ plane parallel atmosphere solution modified to the situation where the incoming radiation is non-zero at the boundary, the solution of which has already been obtained by Donnison and Williams⁵ as

$$T^4 = \left(\frac{3\tau+2}{4} \right) T_{\text{eff}}^4 - \frac{I_2}{ac} \left(\frac{3\tau-2}{4} \right) \quad (1)$$

where T is the temperature at optical depth τ , T_{eff} is the effective temperature and I_2 is the specific intensity per steradian moving inwards at the boundary of the atmosphere (that is the contribution from the dust shell). If we let T_E represent the temperature by which the back radiation alone would raise the temperature of the star then we have

$$I_2 = acT_E^4 \quad (2)$$

Substituting this expression for I_2 into equation (1) gives

$$T^4 = \left(\frac{3\tau+2}{4} \right) T_{\text{eff}}^4 - \left(\frac{3\tau-2}{4} \right) T_E^4 \quad (3)$$

which reduces to the standard Eddington solution if $T_E = 0$.

For young stars the main source of opacity in the atmosphere is negative hydrogen ions, a convenient form of the opacity law being⁶

$$\kappa = \kappa_0 P^{1/4} T^4 \quad (4)$$

P being the pressure and κ_0 a known constant. The condition of hydrostatic equilibrium in the atmosphere combined with the temperature given in equation (3) yields

$$P^{3/2} = \frac{2g}{\kappa_0} (T_{\text{eff}}^4 - T_E^4) \ln \left[\frac{3\tau}{2} \left(\frac{T_{\text{eff}}^4 - T_E^4}{T_{\text{eff}}^4 + T_E^4} \right) + 1 \right] \quad (5)$$

g being the surface gravity of the star.

The usual Schwarzschild⁶ condition for the onset of convection requires that

$$\frac{T}{P} \frac{dP}{dT} = \frac{5}{2} \quad (6)$$

Substitution of the derived expressions for P and T in this equation show that convection sets in at an optical depth τ_c given by

$$\tau_c = \frac{2}{3} \left[\frac{T_{\text{eff}}^4 + T_E^4}{T_{\text{eff}}^4 - T_E^4} \right] (e^{16/15} - 1) \quad (7)$$

Convective stars behave like polytropes of index 3/2 and so the pressure and temperature are known in the interior. At the boundary between the interior and the atmosphere these values must agree with those derived from the atmosphere models. Setting these values equal yields the equation

$$(T_{\text{eff}}^4 + T_E^4)^{15} (T_{\text{eff}}^4 - T_E^4)^{16} = A M^{28} R^4 \quad (8)$$

A being a known constant.

The luminosity of the central star is given by

$$L = \pi a c R^2 T_{\text{eff}}^4 \quad (9)$$

and this energy output has to be provided by the gravitational contraction of the star and the incoming energy from the shell. For an object of polytropic index 3/2 this gives

$$L dt = - \frac{3}{7} \frac{G M^2}{R^2} dR + \pi a c R^2 T_E^4 d\tau \quad (10)$$

The time taken for contraction from an initial radius R_0 is given by substituting for L from equation (9) in equation (10) which yields

$$t = - \frac{3G}{7\pi a c} \int_{R_0}^R \frac{M^2 dR}{R^4 (T_{\text{eff}}^4 - T_E^4)} \quad (11)$$

If we assume that λL is the luminosity which is re-emitted by the shell on to the star then $\lambda = (T_E/T_{\text{eff}})^4$, so that equations (8) and (9) become

$$T_{\text{eff}}^{124} = \frac{A M^{28} R^4 (1 + \lambda)}{(1 - \lambda^2)^{16}} \quad (12)$$

$$L = \frac{\pi a c A^{1/3} M^{1/31} R^{66/31} (1 + \lambda)^{1/31}}{(1 - \lambda^2)^{16/31}} \quad (13)$$

while the integral in equation (11) can be evaluated to give

$$t = \frac{93 G M^{34/31}}{679 \pi a c A^{1/31}} \left(\frac{1 + \lambda}{1 - \lambda} \right)^{16/31} \left(\frac{1}{R^{97/31}} \text{ and } \frac{1}{R_0^{97/31}} \right) \quad (14)$$

For a thin shell some of the radiation will pass completely through, so that only part will be absorbed by the shell and re-emitted isotropically. In the limit, when the shell is just thick enough so that no radiation passes through unabsorbed, λ will have the value $\frac{1}{2}$, while for all thinner shells less than half the radiation will return. From equation (12) we can see that for a thin shell the maximum increase in the effective temperature of the central star for a given radius is $[2^{31/315}]^{1/124} = 1.04$, a fairly minimal increase.

Similarly, the luminosity of the star will increase by 1.16, again not a very substantial increase, while the contraction time

is also increased by only 1.7. A thin shell will therefore cause no significant change in the structure and evolution of the central star.

For a very thick shell the proportion of radiation returned can be considerably greater than a half, though presumably λ can never actually be unity as some radiation will always be re-emitted outwards. From the observations of possible circumstellar shells (Low *et al.*⁷, Wickramasinghe and Nandy⁸) the average emission temperature is ~ 600 K, while presumably their inner surfaces must be limited to maximum temperatures of $\sim 3,600$ K, the temperature at which all types of grains will melt (Weast⁹). The ratio of radiation escaping from the system to that returning to the star can therefore be as low as $(600/3,600)^4$ so that

$$\frac{1 - \lambda}{\lambda} \approx \frac{1}{6^4}$$

If we substitute this value for λ into equations (12) to (14) we find that the luminosity rises dramatically by a factor of ~ 29 and the corresponding evolutionary time by ~ 45 . This means that the gravitational contraction now only has to supply energy at $\sim 1/45$ th of the previous rate, so that most of the star's energy comes from what is returned to it by the thick shell. The presence of a thick shell therefore has quite a substantial effect on the star.

Of course, while such a dust shell is there, we cannot see the star, as we have outlined earlier, but instead see the dust shell emitting in the infrared. Once the dust shell clears, however, we see the star in a position and with an age which is considerably different from that which it would have had if it had evolved without back warming from the dust shell.

The effects of back warming by a dust shell will therefore depend very much on the thickness of the shell, with a thin shell having very little effect so that for any shell where some light from the parent central star is observed there will be no significant effect. A thick shell, however, will mask the main characteristics of the star and substantially slow down its evolution.

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Constraints on the nature of the ancient lunar magnetic field

MANY lunar rocks possess a stable component of natural remanent magnetisation (NRM) with specific intensities around 10^{-6} e.m.u. g^{-1} (refs 1-6). Most of them also have compaction ages of more than 3×10^9 yr (refs 5 and 6). Ancient lunar surface magnetic fields of the order of 10^{-2} -1.2 gauss have been postulated to explain the observed NRM in lunar rocks¹⁻⁶, and several interesting suggestions have been made concerning the origin of such high magnetic fields during the early history of the Moon. Runcorn and Urey⁷ have proposed an intrinsic dipolar field, acquired by the Moon during its

initial stages of formation. Such a dipolar field could result either from a primaevial magnetisation of the Moon by an external field or from the action of an ancient lunar dynamo. Collinson *et al.*⁵ supported that theory with the observation that the palaeofield required to explain the stable NRM in lunar rocks decreases with the decreasing formation or compaction ages of the rock samples. I point out here some constraints that can be put on the origin and nature of the ancient lunar magnetic field, based on the observation of solar-wind ions in lunar breccias with compaction ages of more than 3.2×10^9 yr.

Considering a lunar dipolar magnetic field, the critical dipole moment required to stop the incident solar-wind ions from reaching the lunar surface is $\sim 2.5 \times 10^{21}$ gauss cm³, which corresponds to an equatorial surface field strength of $\sim 4 \times 10^{-4}$ gauss. This value has been obtained simply by equating the oppositely directed solar wind dynamic pressure and the magnetic field pressure near the lunar surface. The solar-wind ions are assumed to be incident at right angles to the direction of the lunar dipole axis. The average velocity, v , and number density, n , of solar-wind ions used in this calculation are taken from contemporary solar-wind data as 500 km s^{-1} and 5 cm^{-3} , respectively.

The critical (cutoff) dipole moment and surface field are directly proportional to the product of the velocity and square root of the number density of the solar-wind ions. This dependence is shown in Fig. 1 for a hypothetical range of the parameter $v\sqrt{n}$ which includes the contemporary value; also shown is the predicted lunar surface field 3.2×10^9 yr BP (ref. 5). Though this assignment is somewhat arbitrary when all the available data on lunar magnetism are considered (see ref. 6 for a detailed discussion), the data do indicate the presence of an ancient lunar surface magnetic field greater than a few times 10^{-2} gauss, up to a maximum of 1.2 gauss during the period $3.0\text{--}4.1 \times 10^9$ yr BP (refs 5 and 6). Thus, it is obvious from Fig. 1 that if the number density and velocity of the solar-wind

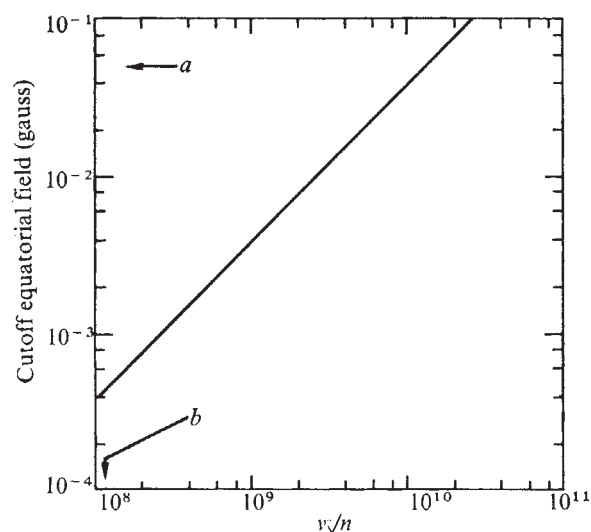


Fig. 1 Cutoff lunar surface magnetic field required to stop incident solar-wind nuclei from reaching the lunar surface, as a function of the parameter $v\sqrt{n}$, where n is the number density (cm^{-3}) and v the velocity (cm s^{-1}) of the solar-wind ions. *a*, Predicted lunar surface field 3.2×10^9 yr BP (ref. 5); *b*, contemporary solar wind ($n = 5 \text{ cm}^{-3}$; $v = 5 \times 10^7 \text{ cm s}^{-1}$).

ions have remained nearly the same throughout the last 4.0×10^9 yr, then the predicted field at the lunar surface more than 3.2×10^9 yr ago is more than sufficient to stop the incident solar-wind ions from reaching the surface.

These considerations, therefore, imply that solar-wind ions should not be observed in individual components of lunar breccias with compaction ages $\geq 3.2 \times 10^9$ yr. As shown in

Table 1 Data from lunar breccias

Sample No.	Type	^{36}Ar ($\text{cm}^3 \text{ STP g}^{-1}$) $\times 10^{-6}$	Compaction age $\times 10^9$ yr
14318	Spherule-rich microbreccia	27(9)*	$3.69 \pm 0.09(8)$
14301	Microbreccia with abundant chondrules	61(10)	$\sim 3.7(10,11)$
14313	Polymict breccia	360(10)	$> 3.2(?) (10)$

*Reference numbers in brackets.

Table 1, however, there exists at least one lunar breccia (14318) which has an extremely well defined $^{40}\text{Ar}\text{--}^{39}\text{Ar}$ compaction age of $3.69 \pm 0.09 \times 10^9$ yr and is also enriched in solar-wind ion concentration^{8,9} (using ^{36}Ar concentration as an indicator of trapped solar-wind ions). In addition, individual components of lunar breccias 14301 and 14313, enriched in solar-wind ion concentration¹⁰, were probably compacted more than 3.2×10^9 yr BP (refs 10 and 11). These experimental observations, therefore, apparently preclude the presence of an intrinsic lunar dipole field during the early history of the Moon, producing a surface field of more than 3×10^{-2} gauss. It is possible, however, that, given certain constraints on the mode of origin and the nature of the ancient lunar magnetic field, both the presence of a large lunar magnetic field more than 3.2×10^9 yr ago, and the observations of solar-wind ions in lunar breccias with compaction ages of more than or around 3.2×10^9 yr, can be explained.

Using the model of a global lunar dynamo several alternatives allow the solar-wind ions to reach the lunar surface. First, frequent reversals of the lunar dipole field could occur, producing a long-term field that is nearly zero. These occur in the case of the geomagnetic field, though there is no evidence yet for similar lunar field reversals. Alternatively, a wandering of the lunar dipole axis, could, in principle, allow solar-wind ions to reach different regions of the lunar surface by streaming along the polar direction. Recent analyses of the magnetic fields over the lunar surface indicate that the ancient lunar magnetic dipole axis may not have been oriented along the present rotation axis of the Moon¹³. It is highly questionable, however, whether this evidence by itself can be taken to imply a wandering of the lunar dipole axis. A third remote possibility is a late onset of the dynamo action, such that the individual components of the lunar breccias received their solar wind irradiation before a magnetic field became established. The observed stable component of NRM in lunar samples 62235 and 77017, however, requires that the onset of the lunar magnetic field must have taken place not later than 4.1×10^9 yr BP (ref. 6).

Models invoking external field magnetisation as the cause of the ancient lunar magnetic fields constrain the dipole axis along the rotation axis, so field reversals are not possible. Further, because of the large magnitude of the external field (~ 20 gauss) required by such models, it has been proposed⁷ that such conditions can only have been fulfilled during the very early stages of the Solar System, more than 4.5×10^9 yr BP.

Finally, considering a local field model¹², none of the constraints required in the global dynamo model are necessary to explain the observation of solar-wind ions in old lunar breccias. Since the trajectories of the solar-wind ions in a local magnetic field depend on the magnitude, scale length and direction of the field¹⁴, there always exists the possibility that the solar-wind ions may reach the lunar surface. Further, the absence of a perfect correlation between the inferred magnetic field strengths at the lunar surface and the compaction or formation ages of the rock samples^{5,6} can also be explained within the framework of the local field hypothesis. The greatest drawback of the local field hypothesis is the absence of a precise model concerning its origin. One of the models proposing the presence of 'fescons', small pockets of Fe-FeS

It is implicit in this discussion that the physical properties of the solar-wind ions have remained the same throughout the last 4×10^9 yr or more. As noted already, the critical surface field strength of the lunar magnetic field required to stop the incident solar-wind ions from reaching the lunar surface is directly proportional to the parameter $v\sqrt{n}$. Therefore, if the value of this parameter was at least two orders of magnitude higher 3.2×10^9 yr ago than today, the solar-wind ions could still have reached the lunar surface even if the lunar surface magnetic fields were $\sim 3 \times 10^{-2}$ gauss (see Fig. 1). Assuming that the velocity of the solar-wind ions has remained the same, such an increase in $v\sqrt{n}$ implies an increase of more than four orders of magnitude in the number density of the solar-wind ions over the contemporary value. Although a case can be made for an enhanced solar wind flux during the very early active phase of solar evolution, close to 4.6×10^9 yr BP, it is highly unlikely that this will be the case at around 4.0×10^9 yr BP or later, times relevant to this discussion. Higher orders of magnitude cannot be advocated for the velocity of the solar-wind ions for a similar reason. Further, rare gas analyses of the gas-rich achondrite Khor Temiki have conclusively shown¹⁶ that the range of solar-wind ions in silicate minerals of this meteorite is $\leq 2,000$ Å, corresponding to an energy of ~ 1 keV/a.m.u. Since the solar wind irradiation of the Khor Temiki meteorite predated its compaction age, which is $> 4.0 \times 10^9$ yr, as indicated from preliminary data (D. Leich, personal communication), it can be safely concluded that the velocity of the solar-wind ions has remained roughly the same during the past 4×10^9 yr.

In conclusion, observations of solar-wind ions in individual components of lunar breccias with compaction ages of more than 3.2×10^9 yr rules out the hypothesis that external field magnetisation initiated the ancient lunar magnetic field. This hypothesis is also unsuitable for other reasons concerning lunar magnetism^{6,13}. The lunar dynamo model is a possible alternative only if an *ad hoc* assumption like field reversal is introduced. A strong case cannot be made for the local dynamo model¹² as it is yet to be fully developed both from an analytical and an experimental viewpoint.

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Longitudinal dependence of ionospheric total electron content in the South Atlantic Geomagnetic Anomaly

WE present here a report on the changes with longitude of the total ionospheric electron content (TEC) found in the region known as the South Atlantic Geomagnetic Anomaly (SAGA). These have been investigated by measurements of the phase delays of the modulation of transmissions from the ATS-6

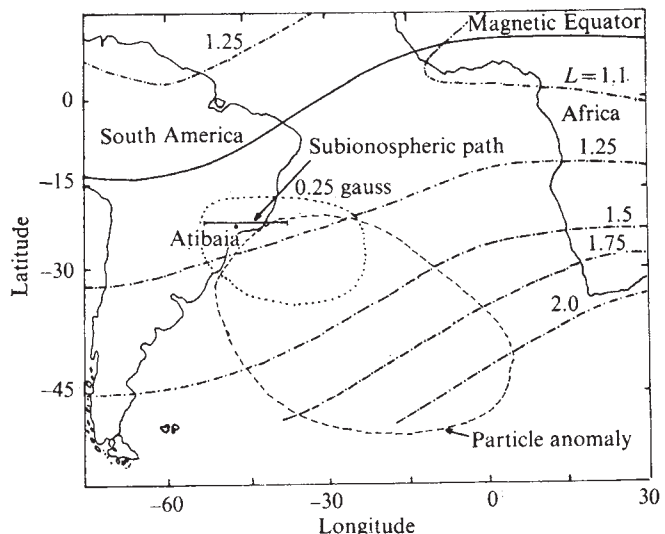


Fig. 1 Simplified map of the South Atlantic anomalous area. It shows the satellite's 420-km altitude subionospheric path, the 0.25-gauss magnetic contour at the Earth's surface, the L parameter calculated for 500 km altitude, and the contour for the 'particle South Atlantic Anomaly' after Ginzburg *et al.*¹³.

radio beacon^{1,2}. The ATS-6 geostationary satellite moved from a position of 94°W to 34°E , between May 23 and June 21, 1975, thus providing the opportunity to investigate this longitude dependence.

Our method of determining the TEC, based on the group delay technique, is insensitive to the values of the geomagnetic field, for the v.h.f. region^{3,4} (140 MHz and 360 MHz, with a 1-MHz modulation frequency), and the electron content includes the ionosphere and the plasmasphere. The TEC can be determined from the simplified equation

$$fNds = 2.7 \times 10^{16} \Delta\phi \text{ m}^{-2}$$

which is derived from well known formulations^{1,5}, s (m) is the ray path from the satellite to the receiving station, N (m^{-3}) is the electron concentration, and $\Delta\phi$ (rad) is the measured group delay. The reported TEC values refer to $\int Ndh$, where $h = s/\sec \chi$, and χ is the satellite direction's zenith angle at the subionospheric point situated at an altitude of 420 km.

ATS-6 transmissions were picked up with two circularly polarised antennae at Itapetinga Radio Observatory, Atibaia, São Paulo, Brasil (latitude $-23^\circ 11' 03''$, longitude $46^\circ 33' 48''$ W). The satellite was tracked from 94°W to 7°E , corresponding to a 420-km altitude subionospheric path of 53°W to 40°W , at a latitude of $\sim -21^\circ$, as shown in Fig. 1. Observations beginning May 3, 1975, were also carried out for several days before the satellite's movement. With the exception of May 19–20, for which some geomagnetic activity was reported, the entire period covered in this experiment was extremely quiet, both magnetically and in terms of solar activity⁶. In Fig. 2 we show the superimposed TEC diurnal values reduced to the subionospheric point vertical ($\int Ndh$), before the satellite movement (a), and over the days of satellite movement (b). Higher values were obtained before the satellite movement, at a subionospheric longitude of 53°W . Averages and s.e.m.s for this longitude were, at maximum (just after local noon 17–18 UT) $(29.6 \pm 1.3) \times 10^{16} \text{ m}^{-2}$, and at minimum (the pre-dawn hours from 6–7 UT) $(4.7 \pm 0.7) \times 10^{16} \text{ m}^{-2}$. These values are comparable with values obtained at Nairobi, near the Equator, but outside SAGA, during quiet Sun conditions, by the Joint Satellite Studies Group⁷, using the Faraday rotation technique for TEC determinations.

In Fig. 3 we indicate the TEC values as a function of the subionospheric longitude where the transmissions were received.

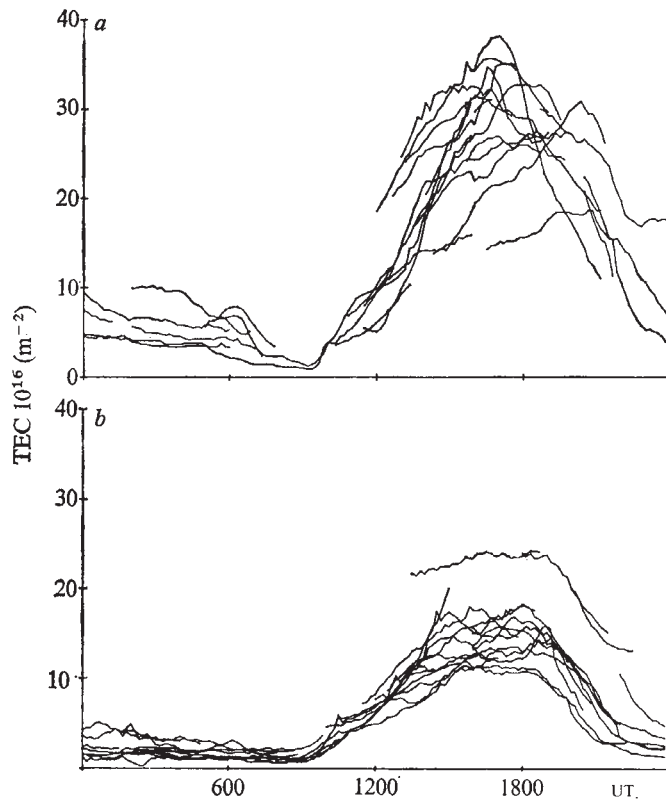
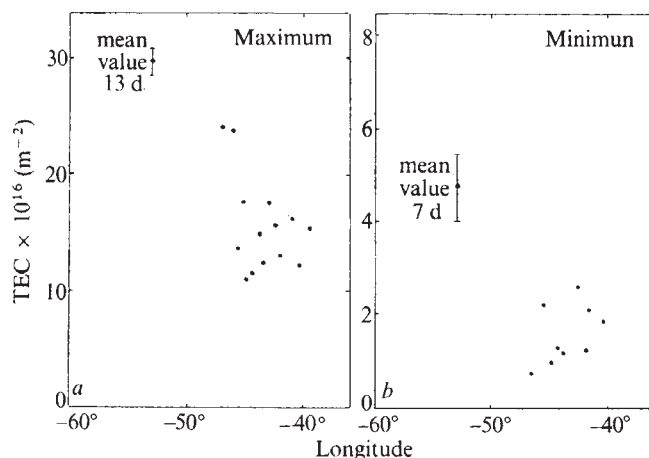


Fig. 2 Daily plots of the total electron content (TEC) in UT (LT is UT-3 h). *a*, For the days before the satellite movement—a subionospheric longitude of 53°W. *b*, The days when the satellite was moving east. The two abnormally high values in (*b*) come from satellite positions before 46°W, as shown in Fig. 3*a*.

The first value, at 53°W, corresponds to the average of the period previous to the satellite movement. The transmissions were very poor during the first days of movement, and useful information was obtained only from 53° to 47°W. A very substantial reduction of the TEC values was then obtained in the longitude range of 46°W–40°W, both at daytime maximum (Fig. 3*a*) and at pre-dawn minimum (Fig. 3*b*). Average values for the 46°W–40°W longitude range were of $(14.1 \pm 0.7) \times 10^{16} \text{ m}^{-2}$ at maximum (17–18 UT) and of $(1.7 \pm 0.2) \times 10^{16} \text{ m}^{-2}$ at minimum (6–7 UT), corresponding to reductions in TEC of 52% and 64% compared with the 53°W figures. In Fig. 3*a*

Fig. 3 Longitude dependence of TEC at maximum *a*, and at pre-dawn minimum, *b*. Significant decreases were found when the satellite was eastwards of ~46°W. Note that the first point at 53°W is an average for the days before movement, and the bar is the s.e.m.



there is still a suggestion of a sharp transition in TEC at ~46°W.

The observed change in the TEC cannot be attributed to a seasonal effect, since the entire period of observation covered only 44 d. If we take typical equinox-to-winter midday reduction in TEC, in conditions of small sunspot number⁸, we find that in the period covered by our experiment, the seasonal influence on the TEC reduction should be of about only 6%. On the other hand, we do not think the observed changes in TEC could be attributed to the dependence of electron concentration on the magnetic dip angle effect^{9,10}. The variation of longitude from the subionospheric point corresponded to a change in dip angle from -18° to -26° (ref. 11), too small to produce a significant change in the TEC.

All the 420-km altitude subionospheric path observed is situated within a 0.25-gauss magnetic contour (at the Earth's surface), corresponding to the so-called Brazilian Geomagnetic Anomaly. Our results suggest that the effect on TEC is displaced eastwards from that region. This is consistent with Roederer's¹² interpretation of the effect produced by the eccentric Earth magnetic dipole position, displacing the trapped particle shell eastwards over the Atlantic Ocean. This 'particle anomaly' has been described, for example, by Ginzburg *et al.*¹³. It shows higher fluxes of charged particles, as measured by satellite counters in the altitude range 187–339 km (as was also indicated in Fig. 1). In a more recent study Torr *et al.*¹⁴ have shown that in this area there are increased precipitated electron and proton fluxes. They find a sharp rise in the proton flux for an *L* shell value of 1.5 at ~30°W, and in the electron flux at *L*=2 at about the same latitude.

Interpretation of aeronomic phenomena in SAGA is difficult and often controversial^{15,16}. Reduction in electron concentration in the Anomaly has been already suggested by Torr and Torr¹⁷, as a net result from excess corpuscular bombardment of the upper ionosphere, producing additional ionisation, and also temperature increases, increasing the scale height and the loss rates. Indeed, Taylor¹⁸ has shown that for an F-region trough (that we might take as a similar effect in a short time interval) a significant reduction of electron concentration occurred while increases in the electron and ion temperatures were observed.

One possible explanation for the decrease of the TEC in the SAGA is that the velocity terms in the continuity equation^{19,20} for electrons are important

$$\frac{1}{N} \frac{\partial N}{\partial t} = -\frac{1}{N} \nabla \cdot \mathbf{V} N - \nabla \cdot \mathbf{V}$$

where $\mathbf{V} = (\mathbf{E} \times \mathbf{B})/B^2$ is the electromagnetic drift velocity that arises from the magnetospheric dawn-to-dusk large scale electric field *E*, which can have an important effect on the motion of plasmasphere low energy charged particles²¹. The total divergence $\nabla \cdot \mathbf{V}$ is usually negative²⁰, although it may depend on assumptions made about the motion *V* when the ion drag effect is important¹⁹. Thus the proposed explanation could be tenable only if the first term on the RHS of the above equation is positive and sufficiently large. This may be true within the SAGA as a result of inherent gradients of *N*. Further quantitative researches are suggested for the establishment of an adequate model for explaining the observed TEC reductions in the Anomaly.

Diurnal TEC measurements of Faraday rotation taken in the same period confirm diurnal change reduction as the satellite moved eastwards. Another study relating the two methods of TEC determination is in preparation.

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Hudson Bay, Lake Zissaga and the growth of the Laurentide Ice Sheet

THE Laurentide Ice Sheet, by far the largest of the Northern Hemisphere continental glaciers, has formed and disappeared several times during the Quaternary¹. I suggest here that, at the times of its formation and early growth, the mass balance of the ice sheet depends strongly on the physical behaviour of Hudson Bay, which is in turn largely controlled by the growing ice. Rapid changes in the size, salinity, and ice and snow cover of Hudson Bay must all have important effects on the mass balance of the growing Laurentide Ice Sheet.

Ives *et al.*¹ have advocated the concept of 'instantaneous glacierisation', whereby continental glaciation in the Northern Hemisphere is initiated by the appearance of perennial snowfields on Baffin Island and in northern Labrador, and they present considerable field evidence in support of their views. I have taken the same position on theoretical grounds²; a region with a high albedo absorbs relatively little energy from the Sun, but constantly loses energy as outgoing infrared radiation. It thus has an energy deficit that can only be balanced with advected energy from the surrounding area. If a significant fraction of the energy available for advection is in the form of latent heat, and if the region is cool enough so that precipitation generally freezes, more snow will fall each year than melts, and the snowfield will grow into an ice sheet.

Because of its position, Hudson Bay must be an important source of moisture for the formation of perennial snowfields on Baffin Island and in northern Labrador and for their initial growth; at present, snow falls during all seasons, and the highest snowfall occurs during autumn and early winter, before Hudson Bay freezes^{3,4}. Once ice sheets form and begin to grow, the sea level will fall, however, and this will decrease the size of Hudson Bay and its

effectiveness as a moisture source. This negative feedback mechanism will retard or reverse the growth of the ice sheets, leading either to steady-state ice sheets or perhaps to systematic oscillations in ice sheet size.

This feedback relationship will operate only while Hudson Bay is an arm of the ocean. A growing ice sheet on Baffin Island will, however, eventually form an ice dam that will block Hudson Strait, as a study by Mahaffy⁵⁻⁷ has suggested. This would transform Hudson Bay into an immense proglacial lake and alter its size, salinity, and direction of drainage. This lake is designated here as Lake Zissaga (from an Indian word for the onset of winter).

With Hudson Strait blocked by ice, Lake Zissaga will increase in size and decrease in salinity until it finds an outlet, flooding low lying land and eventually draining to the south through the Great Lakes. These changes will act in two ways to intensify the developing glaciation, depending on whether Lake Zissaga is frozen or not.

If the lake is not frozen, its increased size will make it a more effective moisture source for the developing ice sheet, and this will lead to renewed or augmented growth of the ice sheet kernels on Baffin Island and in northern Labrador, which can draw moisture from the lake to help balance their local energy budgets using latent heat.

If Lake Zissaga is frozen, it will no longer be an effective moisture source for the ice sheet kernels in the north, but it will have a high albedo and a large deficit in its local energy budget, which, as noted above, must be balanced using energy advected from the surroundings. If this advected energy includes large amounts of latent heat and therefore produces high snowfall, Lake Zissaga will become permanently ice covered and will become a new centre of growth for the Laurentide Ice Sheet.

There are several reasons why the permanent freezing of Lake Zissaga is not unlikely. Oceanographic data, although sparse, suggest that Hudson Bay now has an energy deficit of a few percent that is balanced either by movement of relatively warm water into Hudson Bay from the Atlantic, or by a net export of ice⁸. Neither of these mechanisms would operate for Lake Zissaga. Freezing would also be aided by the low salinity of the lake, as fresh water both freezes and melts at higher temperatures than salt water⁹. This would cause Lake Zissaga to remain frozen for a longer period each year than Hudson Bay does, and would increase the regional energy deficit because of the effect of the ice on the albedo. Yet another effect is that the inflow of warm water from the surrounding land areas would be reduced, because of the flooding of low areas and the consequent reduction in land area draining into the lake. For all of these reasons, it seems likely that Lake Zissaga, once it formed, would have a strong tendency to freeze over and stay frozen.

Permanent freezing of Lake Zissaga will result in a sudden southward shift in the area of maximum snow accumulation on the growing Laurentide Ice Sheet. It will extend the area with a large energy deficit much closer to the Gulf of Mexico, which is an important source of warm, moist air masses that nourish the growing ice sheet while balancing its regional energy budget. This southward shift will significantly change the relationship between the growing ice sheet and the oceans from which it derives both moisture and energy. The magnitude of the total energy deficit over the ice sheet will increase considerably, and at the same time the energy gradient between the ice sheet and the oceans will intensify, both because of the increased total deficit and because of the decreased distance between them. These changes will increase the rate of accumulation of snow on the ice sheet, and they may also alter the oscillation period between stadial and interstadial periods.

Another possibility is that the ice dam may be somewhat unstable during its early phases, so that the accumulation of water in Lake Zissaga could cause the dam to break

before the lake freezes to the bottom. If the ice cover were floating at the time the dam broke, much of the ice could be swept away during the draining of the lake; this would restore a low surface albedo and eliminate the local energy deficit required to drive a continental glaciation. Such a mechanism provides a plausible, although not yet proven, way to account for the short but dramatic global coolings that Flohn¹⁰ has termed "abortive glaciations".

The concept of 'instantaneous glacierisation' may thus be extended to include not only the initial formation of ice sheet kernels on Baffin Island and in northern Labrador, but also the freezing of Lake Zissaga and its sudden transformation from a large proglacial lake into a large, thin, growing ice sheet.

If the Laurentide Ice Sheet originated in the North, as Ives and his coworkers have suggested¹, then Lake Zissaga must have existed, and it must have been an important factor in the development and growth of the Laurentide ice. The episodic nature of Northern Hemisphere continental glaciation during the Quaternary must be in part a response to the huge perturbations to the global climatic system introduced by the permanent freezing of Lake Zissaga.

If all of this is true, then local geographical conditions may allow small ice sheets such as those that grew on Baffin Island to produce significant environmental changes far beyond their own margins, and these changes must be understood before we can reach a complete understanding of the dynamics of ice ages.

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Earth tides, volcanos and climatic change

A STATISTICALLY significant correlation has been found between the derivatives of the envelopes of peak tidal stresses at high northern latitudes and the mean temperature of the Northern Hemisphere as reflected in oxygen isotope ratios in the Greenland ice cap. We suggest that variations in tidal stresses on the Earth caused by the Sun and Moon cause changes in the amount of stratospheric dust produced by volcanic activity; this in turn changes the thickness of the stratospheric dust veil and hence the atmospheric radiation balance. We find that, for a simple model, periodic variations in tidal stress account for 13% of the variance in the ice-core temperature record.

The connection between volcanic activity and the stratospheric dust veil is well documented¹⁻³, and a resultant effect on mean global and hemispheric temperatures has been demonstrated²⁻⁴. Thus, an ability to predict volcanic activity would certainly help in predicting climatic changes. We have therefore examined the hypothesis that volcanic activity is triggered by quasi-periodic increases in the amplitudes of the tidal stresses exerted on the Earth.

Tidal forces produce stresses in the Earth's interior and crust. Because of the Earth's rotation and the Moon's orbital motion, any particular place on the Earth's surface experiences two

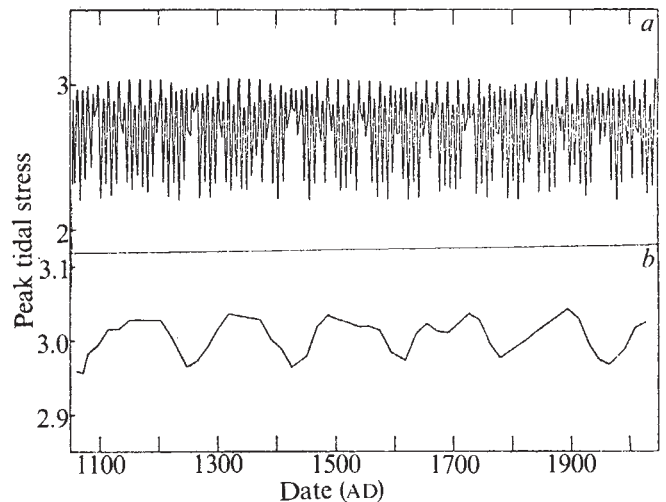


Fig. 1 *a*, Upper envelope of fortnightly tidal stress peaks at 60°N latitude. One unit on the vertical axis represents 4.532×10^{-5} N kg⁻¹; *b*, Upper envelope of Fig. 1*a* with 8.85- and 18.61-yr periods filtered out. Note change in vertical scale.

complete cycles of high and low tidal stress roughly every 25 h. The Earth and Moon follow elliptical orbits whose planes are tilted with respect to each other, and whose orbital elements are changing continuously (particularly those of the Moon). Thus, the amplitude of the basic 12.5-h tidal stress cycle follows complex but completely predictable patterns^{5,6}.

Correlations have been reported between the fortnightly tidal stress peaks caused by the Moon's orbital motion about the Earth and such events as eruptions of Mt Stromboli⁷ and Yellowstone Park geysers⁸. Tectonic activity might therefore be expected to correlate with a tidal period of $P_1 = 8.85$ yr caused by the advance of the longitude of the Moon's perigee ($\tilde{\omega}$) (which determines the times of alignment of the Moon's perigee with the Earth's perihelion) and with the period $P_2 = 18.61$ yr of the regression of the longitude of the node (Ω) of the lunar orbit on the ecliptic (which affects the exactness of the alignment of the Moon's perigee with the Earth's perihelion). There is a commensurability between these two angles, in that the argument

$$\theta = \tilde{\omega} - 2\Omega$$

has a long period variation, P_r , given by

$$1/P_r = 1/P_1 - 2/P_2$$

(minus because the node regresses). The value of P_r from this relation is 179.3 yr, suggesting there should be a long term tidal resonance effect of this period.

Precise values for these periods are as follows, where T is in tropical centuries (36,524.22 d) from 1900.0

$$\begin{aligned} P_1 &= 8.84750 + 0.0000225T \\ P_2 &= 18.61330 + 0.0000200T \\ P_r &= 179.33134 + 0.005511T \end{aligned}$$

Thus, the resonance period was 179.29276 yr in AD 1200 and is 179.33553 yr in AD 1976.

To examine in detail the nature of the effects of this long period in the tidal stress produced on the Earth, we first calculated the semi-monthly maximum stresses over the period AD 1200-1970 at latitude 60°N (the region of greatest northern hemispheric volcanic activity)^{3,9}. The tidal terms for the Moon were expanded to second order in the Earth's radius, and those for the Sun were expanded only to first order (Fig. 1*a*). We then filtered out the 8.85- and 18.61-yr periods by taking suitable envelopes (Fig. 1*b*). The first derivative with respect to

time of this filtered record of the peak tidal stress then gives a measure of the average rate of change of long period stress effects in the Earth's core and crust, as well as the rate of change of the amplitude of any resultant strain, flexure, friction, or other results of stress.

Figure 2 shows the power spectrum of the first derivative of the peak tidal stress for latitude 60°N. Also shown is the power spectrum calculated by Dansgaard and coworkers¹⁰ for temperature changes indicated by variations in the oxygen isotope ratio ($\delta^{18}\text{O}$) record in the Camp Century, Greenland ice core (also for the time span AD 1200–1970). We choose this climatic record because of its consistency, accuracy and time resolution and (perhaps because of its location at high latitude inside the circumpolar vortex) because it appears to be an exaggerated representation of the mean mid-latitude Northern Hemisphere temperature. Peaks with periods near 180 yr appear in both the $\delta^{18}\text{O}$ and 60°N tidal stress derivative curves. Mitchell (personal communication) has pointed out that although the two highest peaks in the $\delta^{18}\text{O}$ power spectrum (180 and 78 yr $\pm$ 5%) can be considered as "statistically significant by the conventional criterion (*a priori* test), they were not statistically significant on the basis of *a posteriori* tests in which the location of these two peaks in the spectrum was not predicted in advance from independent information".

The correlation coefficient between the derivative of tidal stress at 60°N and the $\delta^{18}\text{O}$ temperature curve is 0.37; the probability that no true correlation exists as determined by the Student's *t* test is $\ll 0.005$. The square of the correlation coefficient tells us what fraction of the Greenland ice curve can be 'explained' by the tidal force curve; thus we can ascribe 13% of the variance in the $\delta^{18}\text{O}$ curve to derivative tidal stresses at 60°N. We have also calculated tidal stress derivatives for 30°N and find a strong 179.3-yr periodicity there too, but this periodicity was not found at the Equator.

Figure 3 shows a time series comparison between the $\delta^{18}\text{O}$ temperature curve (smoothed by running means) and the calculated tidal stress derivative at 60°N. In spite of the positive correlation of the two curves, two epochs seem to be anti-correlated (~ 1420 –1530 and 1650–1700). This discrepancy may be caused by the action of climatological variables associated with changes in solar activity, since the two discrepant epochs found here are nearly centred on the two strongest solar activity minima over the entire 770-yr period¹¹.

Although we have found a statistically significant correlation between tidal forces acting on the Earth and the mean temperature of the Northern Hemisphere, demonstration of a deterministic connection between tidal stresses and temperature requires an understanding of the evolution of the stratospheric dust veil as well as the causes of volcanic activity. Although the stratospheric dust veil receives sensible contributions of volcanic dust from explosive volcanic eruptions¹, it seems that the main contributor is sulphur dioxide gas (SO_2) vented from many uncatalogued sources as well as the hundreds of known 'active' volcanos (J. B. Pollack, O. B. Toon, C. Sagan, A. Summers, W. Van Camp, and B. Baldwin, unpublished). This gas reacts in the stratosphere to produce sulphur-bearing aerosols as well as sulphuric acid coatings that increase the optical effects of sub-micron nuclei¹². A thicker sulphurous dust veil reflects more sunlight into space and cools the Earth's surface (ref. 4, and J. B. Pollack *et al.*, unpublished).

Volcanic activity serves as a mechanism to release thermal energy from the Earth's interior. Thus, we can view the Earth as a boiler and the inactive volcano or vent as a sealed valve. Conversion of tidal energy to thermal energy by friction is concentrated at plate boundaries¹³, where almost all active volcanos are found. Thus tidal energy helps heat up the boilers and increase the pressure, while tidal stresses weaken and break the seals. Both of these triggering effects increase during periods of increasing peak tidal stress.

Heaton¹⁴ has shown that shallow, larger magnitude earthquakes tend to be triggered by tidal stresses and Tokarev¹⁵ has

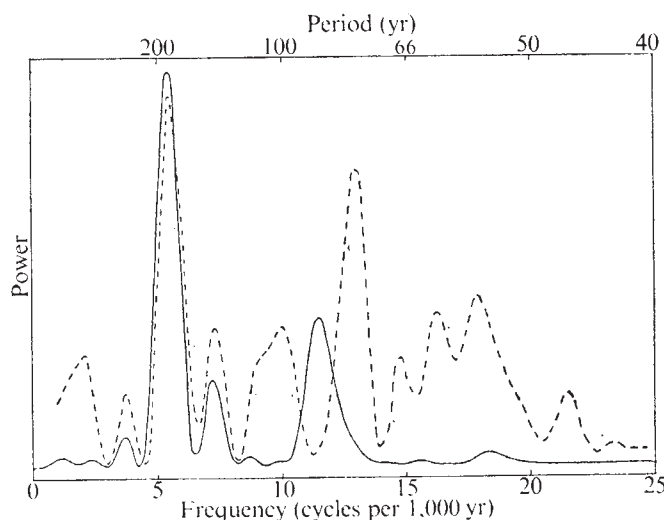


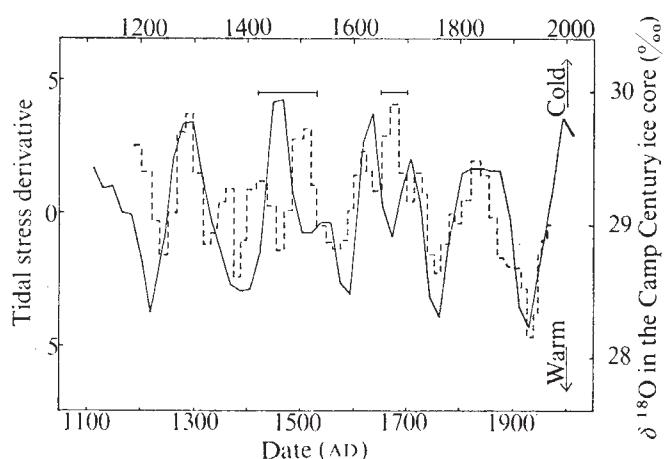
Fig. 2 Power spectra of the $\delta^{18}\text{O}$ Greenland ice temperature curve¹⁰ (— — —) and the derivative of the envelope of the peak vector sum tidal stress at 60°N latitude (—) for the period AD 1200–1970. The peak in the tidal stress derivative at 179.3 yr arises from variations in the Moon's orbital parameters.

found that such earthquakes precede the eruptions of andesitic and dacitic volcanos and outbursts of adventive craters. Thus there seems to be firm support for the belief that tidal forces can trigger volcanic eruptions.

Tokarev has also found that the growth of cumulative seismic strain release before andesitic volcanic eruptions generally follows a hyperbolic curve on a time scale of weeks to months. Although the time scales involved in our work are much longer, we hypothesise that a similar trend is effective in triggering activity in the assemblage of climatically important volcanos. In other words, once a volcano has erupted, its susceptibility to triggering remains low for a longer period of time and then increases rapidly following a hyperbolic or exponential curve. Thus the available stress can exceed the stress required for triggering during periods when the available stress is not decreasing rapidly. Therefore, tidal stress triggering of eruptions occurs preferentially when the value of the derivative is increasing. Since our argument is a statistical one, we are not prepared to speculate on the details of the geophysical mechanism(s) involved.

Even though a statistical relationship has been found between tidal stresses and climatic change, there are ample reasons to

Fig. 3 Derivatives of the envelope of the peak vector sum tidal stress at 60°N (—) and three-point running means of the $\delta^{18}\text{O}$ Greenland ice temperature curve (— — —). Periods of minimal solar activity¹¹ are marked with horizontal bars. One unit on the vertical axis equals $4.532 \times 10^{-5} \text{ N kg}^{-1} \text{ d}^{-1}$.



expect differences between the $\delta^{18}\text{O}$ curve and the tidal stress curve. They include:

(1) The temperature curve used here is only a proxy record of what the instrumentally determined mid-latitude temperature would be. Various proxy records disagree³.

(2) This analysis uses tidal force calculations from only one latitude. A more complete investigation would include integration over all latitude belts with high resolution near the Equator and weighting determined from measured dust and SO_2 emission rates.

(3) The correlation technique used here implicitly assumes a linear relationship between temperature and tidal stress. The true relationship certainly must take some more complex functional form.

(4) Other physical causes for climatic change are also at work. In particular, climatic influences ascribed to solar variability have been widely discussed¹⁶.

(5) Feedback effects are not considered here. They may be very important over fairly long time scales¹⁷.

We have presented evidence that at least 13% of the variance in the $\delta^{18}\text{O}$ temperature record in the Greenland ice cap might be ascribed to periodic variations in tidal stress. We suggest that the 180-yr periodicity found in the $\delta^{18}\text{O}$ record is caused by regular changes in the Moon's orbital motion, with tidal stresses as the physical link. We believe that this mechanism acts along with variations in solar and geomagnetic activity^{11,16}, stratospheric ozone (R. J. Angione, E. Medeiros and R. G. Roosen, unpublished) and other as yet undiscovered physical mechanisms to produce deterministic and predictable climatic changes.

The search for physical mechanism by means of correlation analyses is complicated by the fact that the Universe is filled with periodic and quasi-periodic phenomena, many of which might be affecting the Earth's climate. For instance, 180-yr periodicities in solar activity have been suggested by both the planetary theory of sunspots¹⁸ and natural oscillations inside the Sun (C. L. Wolff, unpublished). It is not clear whether this 'coincidence' is fortuitous or whether two or more separate physical mechanisms are acting in concert.

As a test of our tidal force theory we offer a prediction. The 179.3-yr periodicity in tidal stress at high northern latitudes is precisely out of phase with that at high southern latitudes. Since it is generally believed that stratospheric dust from volcanos outside the tropical ozone is not efficiently transported from one hemisphere to the other, we expect that any 179.3-yr cycle found in Antarctic ice cores should be exactly out of phase with that found in the Greenland ice core. This prediction is of course endangered by the comparative sparsity of active volcanos at high southern latitudes⁹.

Since accurate prediction requires that virtually all of the variance be explained, we do not claim here to have achieved a meaningful climatic prediction, but we are encouraged by these preliminary results.

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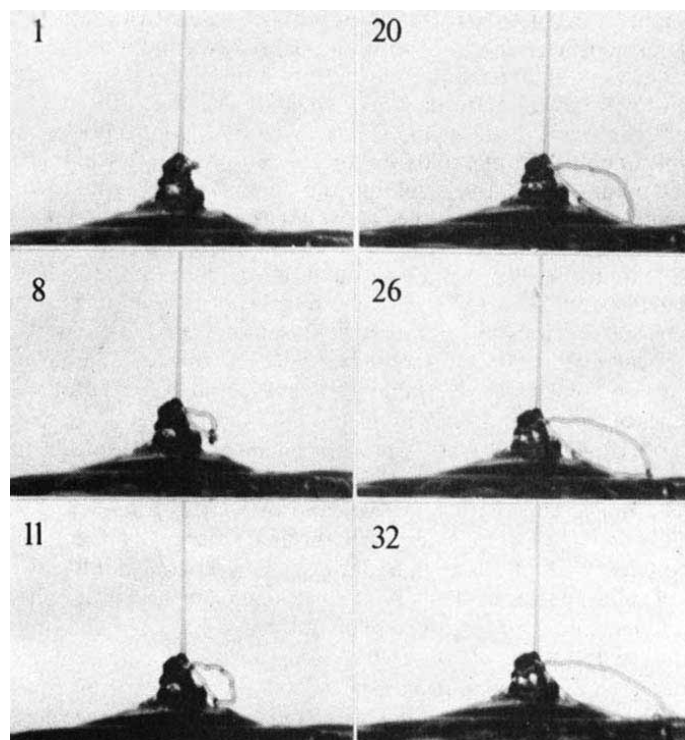
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The Kaye effect revisited

WHEN a thin stream of certain solutions of polyisobutylene in dekaline is poured on to an open evaporating dish, a heap of the liquid forms at the point of contact with the dish. As pouring proceeds a small streamer of liquid emerges from the heap, leaving it at a low trajectory, which gradually steepens before the streamer collapses. The whole effect lasts for about one second¹⁻³. Using a new filming technique and a film speed of 250 frames s^{-1} , we have observed that the emergent streamer is really a loop of liquid, and we here propose a mechanism for this effect. The second frame in Fig. 1 shows the effect. Such looping of the emergent streamer is not unexpected as the liquid is pituitous. In the following frames the loop extends outwards until the bottom of it strikes the surface of the liquid on the flat glass plate. As the streamer is cutting off the liquid from the heap, the heap collapses and flattens, presenting a smaller angle of incidence to the incident stream and causing the bouncing streamer to steepen in trajectory and move azimuthally, as suggested by Kaye. The base of the streamer's trajectory is often as large as 100 mm.

The decay of the streamer may be caused by the elasticity of the liquid being insufficient to maintain such a steep

Fig. 1 The growth of a loop during the build up of a streamer in the Kaye effect. The frames are 1, 8, 11, 20, 26 and 32 from a 250 frames s^{-1} cine film.



¹ *The Eruption of Krakatau, and Subsequent Phenomena* (edit. by G. J. Symons), 405 (Harrison and Sons, London, 1888).

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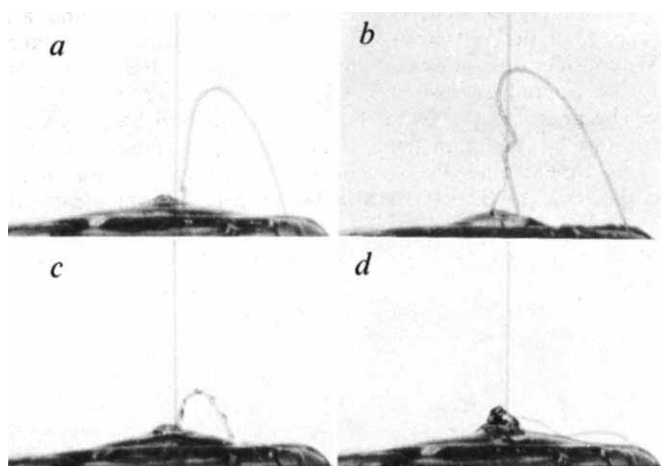


Fig. 2 *a*, The ejection of a kink causing the decay of the Kaye effect; *b*, a collision between the incident stream and the ejected streamer; *c*, coils in the streamer giving a structural weakness and rapid decay; *d*, a secondary streamer caused when the primary streamer strikes the flat glass plate.

trajectory, or by a point of weakness in the streamer, such as a kink that has been ejected from the main body of the liquid (Fig. 2*a*) or, as shown in Fig. 2*b*, by a collision between the streamer and the incoming stream. Once the streamer decays, the heap reforms and the action is repeated.

Some streamers never attain large trajectories and decay after a very short time (~ 0.5 s). This is often because of coiling within the streamer, making it structurally weaker (Fig. 2*c*). It has not been possible to ascertain whether each coil propagates along the streamer, whether each is stationary with respect to the streamer, or whether the motion of the coils is a combination of both.

Figure 2*d* shows a secondary streamer caused by the emergent streamer striking the thin film of liquid lying on the flat glass plate. Such secondary streamers are quite common.

We suggest the following mechanism for the Kaye effect in a polyisobutylene-in-dekalin solution. The liquid in the heap is moving slowly and its viscosity is high, so that it presents a rigid surface to the incoming stream, particularly as it is backed by the flat glass plate. The liquid in the incoming stream is unsheared during its descent and its viscosity too will be high, but on striking the heap there will be rapid changes in the velocity of the liquid on the surface of the heap giving rise to high shear rates. If the liquid were highly shear thinning as well as elastic and pituitous, its apparent viscosity at high shear rate would be low and its elasticity high, the optimum conditions for the reflection of a streamer from the heap.

This proposal requires that the above liquid be highly shear thinning, and it may account for the critical dropping height necessary to provide the best conditions for the Kaye effect. Measurements were carried out on the variation of the apparent viscosity of the liquid with shear rate in the range $40 \text{ s}^{-1} < \dot{\gamma} < 120 \text{ s}^{-1}$ using a concentric cylinder viscometer, the most suitable viscometer available at the time. The results show that the solution is a power law fluid of index 0.4, which, although the means of measurement are not ideal, indicates that the solution is highly shear thinning. It is hoped to repeat this measurement over a larger range of shear rate using a cone and plate viscometer at a later date.

On a macroscopic scale the proposed mechanism for the Kaye effect requires that any polymer solution likely to show it should be elastic, pituitous and highly shear thinning. Moreover, the polymer molecules must have flexible

backbones, but any hydrogen bonding would be disadvantageous because of the additional interactions between the chains involved.

Our present experiments were conducted on a solution of 4.4 g of polyisobutylene in 100 ml of dekalin, the polymer grade being Visnatex L140 of average molecular weight 2×10^6 (Esso). The solution was dropped from a height of 400 mm on to a flat glass plate.

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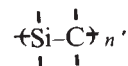
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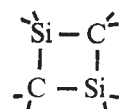
Development of a silicon carbide fibre with high tensile strength

MUCH work has been done on preparing heat-resistant silicon carbide materials in fibrous form, since plastics or metals can be reinforced with them to obtain very heat-resistant material of great mechanical strength. SiC whiskers¹ are, however, impractical because of their shortness (several mm), their non-uniform diameter and high cost of production. SiC-on-W (ref. 2) and SiC-on-C (ref. 3) filaments have been produced by chemical vapour methods. These coated filaments are more expensive, and the treatment for making such composite materials requires careful control. We report here on the synthesis of continuous β -SiC fibres by a new process: the conversion of organometallic polymers to inorganic substances. We have studied the transformation process and the structure and mechanical properties of these fibres.

The synthesis proceeded as follows^{4,5}. Dimethyldichlorosilane $(\text{CH}_3)_2\text{SiCl}_2$, as the starting material, was converted to polydimethylsilane⁶, $[(\text{CH}_3)_2\text{Si}]_n$, by dechlorination with metallic sodium, and was in turn converted to a polycarbosilane polymer,



of mean molecular weight, MW, $\sim 1,200$ by pyrolysis at 400°C in an inert gas atmosphere. The distribution of molecular weights of the polycarbosilane was measured by solvent fractionation. For a number of systems, it was found that the peak of the distribution curve corresponded almost exactly to the MW of the unfractionated polymer. Three calibrated distribution curves are shown in Fig. 1. The peaks of curves *a*, *b* and *c* correspond to MW = 1,086, 1,226 and 1,199, respectively. In our case, it was important for the molecular weight not to be too large. A chemist trying to synthesise a fibre generally desires to produce a polymer chain with a molecular weight from several tens of thousand to $\sim 100,000$. The ring opening polymerisation of disilacyclobutane



does give a giant molecule of molecular weight 10,000–100,000 (refs 7–9) but it cannot be dry- or melt-spun because of its liquid or gummy state. On the other hand, the resinous polycarbosilane polymer obtained from pyrolysis of polydimethylsilanes can be

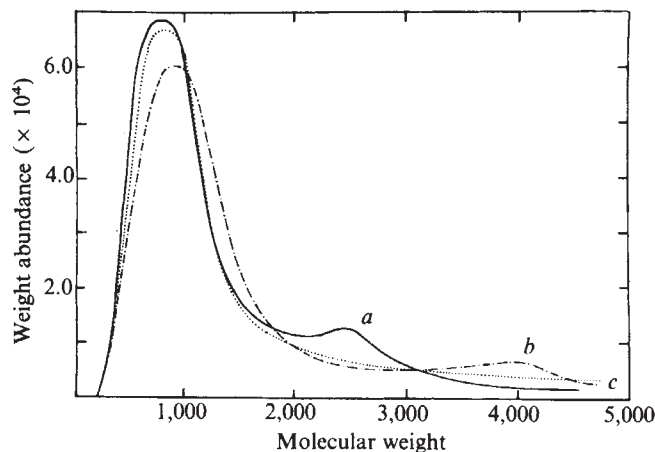


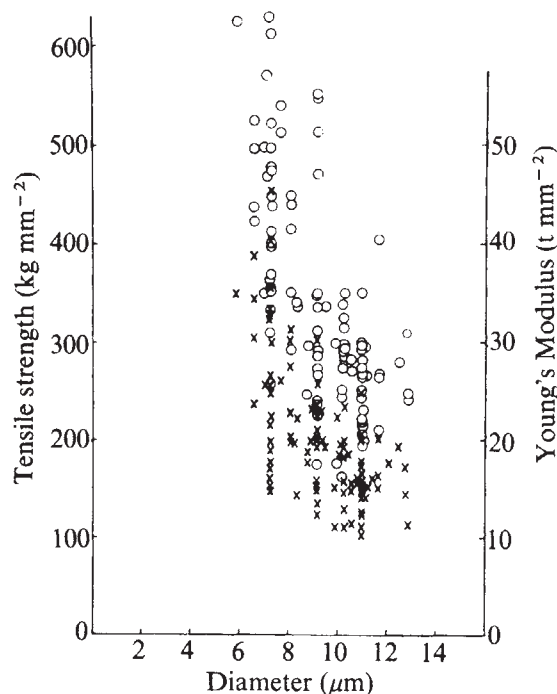
Fig. 1 Calibrated molecular weight distribution curves of polycarbosilanes

melt-spun, and the tensile strength of the starting fibre (8–20 μm in diameter) is very low $(0.5\text{--}1) \times 10^7 \text{ N m}^{-2}$. Heating a bundle of the fibres to $\sim 200^\circ\text{C}$ in air produced a thin silicate film over surface of the fibres; this was done to protect the fibres from melting later on. To break up organic bonds, such as Si-CH_3 and C-H , the polycarbosilane fibre was then heated at up to $1,500^\circ\text{C}$ in vacuum and thereby converted into a continuous SiC fibre with a tensile strength of $62 \times 10^8 \text{ N m}^{-2}$ and a Young's modulus of $4.4 \times 10^{11} \text{ N m}^{-2}$ for a diameter of $\sim 7 \mu\text{m}$.

Infrared spectroscopy and thermogravimetry (TG) of the polycarbosilane from room temperature to $1,300^\circ\text{C}$ indicated that the decomposition occurs gradually above 300°C and is completed at $\sim 800^\circ\text{C}$. Since $\sim 60\%$ of the material by weight is left, this suggests that organometallic and organic bonds, for example, Si-CH_3 and C-H , decompose, so that the polycarbosilane is converted into a material containing mainly Si-C bonds^{4,5}. A little carbon is produced as a by-product, but it is given off with partial oxidation.

The length and diameter of the SiC fibre can easily be controlled by the spinning process. The relation between the diameter of the SiC fibre and tensile strength and Young's

Fig. 2 Tensile strength ($\circ$) and Young's modulus ($\times$) of β -SiC.



modulus is shown in Fig. 2. The values obtained by this method are both higher than those previously reported^{4,5}, and are seen to approach those of β -type silicon carbide whisker. We consider this to be caused by the difference of the preparation in the spun polycarbosilane.

The gradual changes in the X-ray diffraction patterns were measured from room temperature up to the highest temperatures to examine the structural changes going from polycarbosilane to SiC fibre. The broad patterns obtained with

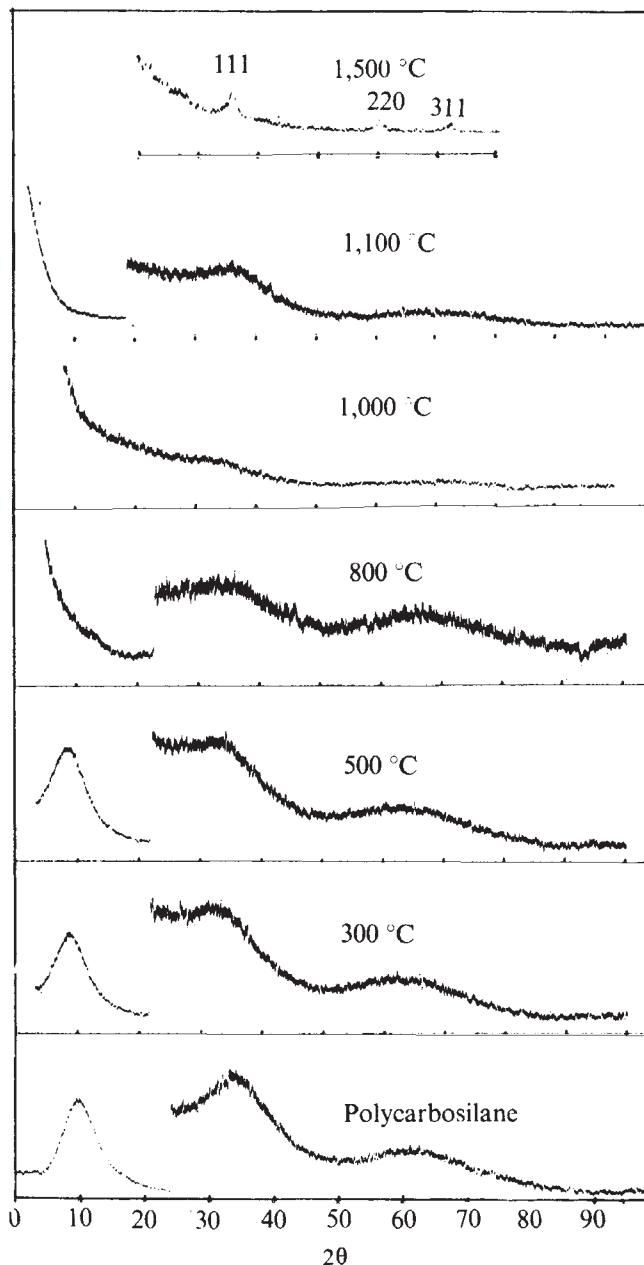


Fig. 3 X-ray diffraction patterns of polycarbosilane and β -SiC fibres heat treated at several temperatures.

$\text{CuK}\alpha$ (Ni filter) are shown in Fig. 3. As the polycarbosilane fibre is heated, the first peak decreases and completely disappears at above $1,000^\circ\text{C}$. The second and third peaks in Fig. 3 decrease for the sample heated at $\sim 800^\circ\text{C}$, but are nevertheless observed for all samples. As the polycarbosilane fibre is heated to higher temperature, those peaks are shifted to a slightly higher angle, the 111 pattern changes, and the 220 and 311 patterns of β -SiC are seen. The polycarbosilane ceases to decompose at $\sim 800^\circ\text{C}$ and is gradually changed into β -SiC when heated to above 800°C . Infrared, TG and

X-ray analysis showed the Si-C skeleton in the polycarbosilane to be converted to the β -SiC structure. From observations with an ultrahigh voltage electron microscope and the X-ray diffraction patterns, we found the SiC fibre heated at 1,500 °C was polycrystalline, made of fine particles of diameter ~ 70 Å. X-ray transmission pinhole photographs of the fibre showed no fibrous texture.

On the other hand, the 30- μ m SiC fibre heated at 1,200 °C was held under a load of 50 g for 3 d in air and showed no sign of breaking. It may well have revolutionary applications as a reinforcing fibre viable up to at least 1,200 °C.

The fine particles of diameter ~ 70 Å cohere tightly to give the fibre. That is, several tens of the polymer molecules (MW 1,200), as described above, assemble to form the inorganic β -type SiC. It is not clear why a high tensile strength should be associated with a SiC fibre composed of fine crystallites, for the tensile strength must be a function of large cohesive forces in the assemblage of fine particles. How can the cohesive force in the assemblage of fine particles be large?

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Low level determinations of environmental cadmium

THE discovery that environmental levels of mercury and lead present serious health hazards has led to intensified investigations into the effects of other heavy metals. Cadmium is a particularly toxic contaminant, and as with mercury, widespread environmental exposure to cadmium is related to its increased technological use¹. We have developed an isotope dilution technique to measure the concentration of cadmium in aqueous solutions with a sensitivity of 0.003 μ g l⁻¹. The concentration of cadmium in two river systems in Western Australia has been measured to establish an accurate set of low level reference determinations in a region which is relatively free of sources of industrial pollution.

Williams and David² have shown that the cadmium content of phosphatic fertilisers and superphosphate used on Australian pastures range from 27 to 90 p.p.m., and that the application of superphosphate is reflected in an increasing cadmium content of the soil. Much of this cadmium is soluble in water and may therefore be transferred to natural waterways. Waterways are of concern because reservoirs may be sited on them and be a source of drinking water and irrigation supplies. The World Health Organization (WHO) has declared that the maximum advisable concentration limit for cadmium in drinking waters is 10 μ g l⁻¹ (ref. 3), whereas the recommended maximum level for irrigation purposes is 5 μ g l⁻¹ for continuous use⁴.

Water samples were collected in high density polyethylene containers since it has been shown⁵ that polyethylene does not adsorb cadmium from aqueous solutions and exhibits a low cadmium blank. A 200-g aliquot of each water sample

was spiked with an accurately known weight of isotopically enriched ¹¹¹Cd tracer. After ensuring that the spike and sample were well mixed, the cadmium was chemically extracted by ion exchange. The extracted samples were analysed in a solid source mass spectrometer, and from the measured isotopic ratios the concentration of the cadmium was determined. Details of the stable isotope dilution technique used in this project are described elsewhere⁶.

Two river systems were selected for study. The Swan River and its tributaries (the Avon, the Helena and the Canning Rivers) form the major river system for Perth and the surrounding metropolitan area. Two major reservoirs—the Canning and Mundaring Weir—are situated on tributaries of the Swan River. The mouth of the Swan River is at Fremantle and for ~ 35 km the river is tidal and salty. The second river system studied in this project was the Peel Inlet System. The Peel Inlet is situated some 50 km south of Perth and is a large body of salt water connected to the sea by a narrow channel. The Murray River and its tributaries flow into the Peel Inlet. The surrounding area is mainly rural and embraces an extensive irrigation district, although in the close vicinity of the Peel Inlet there is a residential area.

Western Australia is a very isolated region, and very little industrial development has taken place in the area covered by this study. The narrow coastal plain is terminated by the Darling Scarp which lies parallel to the coast. This 300-m high escarpment is the western margin of the Yilgarn Block, which is the largest area of Archaean rocks in Australia⁷. The Yilgarn block comprises remnants of metamorphosed volcanic and sedimentary rocks intruded by granitic rocks. The concentration of cadmium in the rock types comprising the Yilgarn Block is ~ 100 ng per g (ref. 8). Most of the region drained by the two river systems is natural forests and farmlands, except for the residential areas around Perth and Mandurah.

The river systems are shown in Fig. 1. The numbers refer to sample locations on the two river systems, details of which are given in Table 1. Altogether 38 water samples were collected from the Swan River System and 14 from the Peel Inlet System. Each sample was analysed in duplicate. The measurements for each region in the river systems have been averaged to give a mean cadmium concentration for each region. The sensitivity of the technique used in this study is 0.003 μ g l⁻¹, and in most cases the accuracy of a single determination is $\pm 10\%$.

The cadmium concentration near the mouth of the Swan River is 0.10 μ g l⁻¹ and is in good agreement with the concentration in normal seawater (0.11 μ g l⁻¹ (ref. 9)). Near Perth the concentration increases to 0.30 μ g l⁻¹ where the

Table 1 Cadmium content of river systems

Sample no. (see Fig. 1)	No. of samples analysed	Locality*	Mean concentration (μ g l ⁻¹)
1	5	Mouth of Swan River	0.10
2	3	Upstream from Perth	0.30
3	9	Middle Swan District	0.09
4	7	Avon River	0.05
5	7	Helena River	0.02
6	7	Canning River	0.05
7	7	Peel Inlet	0.04
8	7	Murray River System	0.05

*The samples were collected in May 1975. There was little rainfall in the 6 months preceding sample collection.

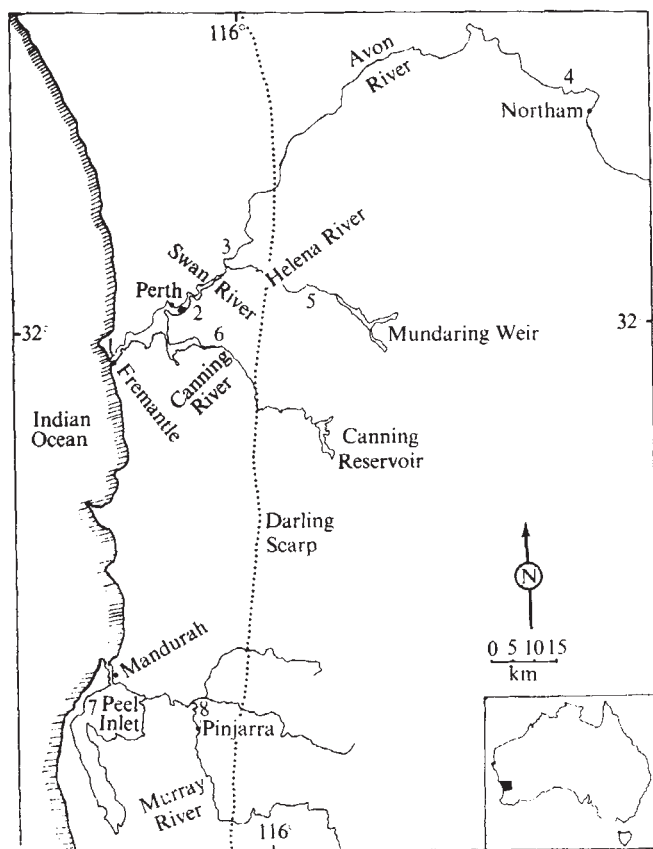


Fig. 1 Sample locations for the Swan and Peel Inlet River Systems in Western Australia.

samples were collected in the vicinity of an industrial area (the maximum value being $0.44 \mu\text{g l}^{-1}$).

The Middle Swan District is situated among vineyards in a semi-rural area and here the cadmium content is $<0.1 \mu\text{g l}^{-1}$. Further upstream the concentration decreases still further, and in the farming area around Northam it is only $0.05 \mu\text{g l}^{-1}$, indicating that the cadmium concentration of this natural waterway is little affected by the presence of fertilisers in the surrounding soil. The Helena River samples were collected near the boundary of a forested catchment area and gave the lowest average value of all the samples of $0.02 \mu\text{g l}^{-1}$. The Canning River is used extensively for recreational purposes, and the surrounding area is residential and semi-rural. The average value is $0.05 \mu\text{g l}^{-1}$, but the individual concentrations are lower upstream.

The Peel Inlet exhibits a low concentration, but this probably arises from the fact that some of the cadmium has been adsorbed on the underlying sediments¹⁰, and reflects the fact that little exchange of water has taken place with the sea. The Murray River system exhibits a slightly higher value of $0.05 \mu\text{g l}^{-1}$, which is consistent with the cadmium concentration in the Avon River in the other farming area near Northam.

The results of this study demonstrate that the cadmium content in the two major river systems in Western Australia is ~ 100 times lower than the WHO limits. The data indicate that the cadmium content tends to decrease upstream from the mouth of the river, and that in the reservoir catchment areas the cadmium content is as low as $0.02 \mu\text{g l}^{-1}$. These values are substantially lower than waterways in other parts of the world¹¹⁻¹³, where the cadmium concentration is often $> 1 \mu\text{g l}^{-1}$. We hope our results will provide a benchmark for the comparison of future measurements on the World's waterways.

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Correlations between concentrations of elements in plants

INTEREST in the cycling of the elements through ecosystems has increased in recent years, and numerous plant species have been analysed. It can be assumed that elements do not cycle independently in vegetation because they are involved in the basic structure and function of cells, which have similar biochemical pathways. Thus concentrations of different elements must be correlated. Little attention has been given to the relationships between concentrations of elements within or across a variety of species. This is undoubtedly because the chemical composition of individuals within a species may vary considerably from one site to another¹. The ratio of different elements may be relatively unaffected by this geographic variation because natural vegetation can absorb and accumulate nutrients selectively² maintaining a balance of intracellular concentrations to optimise metabolism, protein synthesis and tissue production. If concentrations of elements were correlated across a variety of species from different sites, this would indicate a relatively constant ratio between concentrations of elements among plant cells. I report here correlations between concentrations of nitrogen, phosphorus, magnesium, calcium and potassium, across a variety of species, which seem to be based on biochemical similarities between elements in cell metabolism.

Mean concentrations of P, N, K, Ca and Mg in non-vascular plants, vascular plants and aquatic macrophytes were examined for correlations between 54 species. Data were obtained from the literature and unpublished sources, and few restrictions were placed on the data set. Differences in the analytical techniques used were ignored. Sampling times in the studies examined had ranged from April to November but, mostly, analyses had been made on plants collected in summer or autumn. Concentrations were calculated as percentage dry weight.

Non-vascular plants included species of 14 algal genera collected from April to November in Alabama and Mississippi: *Anabaena*, *Aphanizomenon*, *Chara*, *Cladophora*, *Euglena*, *Hydrodictyon*, *Lyngbya*, *Microcystis*, *Mougeotia*, *Nitella*, *Oedogonium*, *Pithophora*, *Rhizoclonium* and *Spirogyra*³. Two moss species collected in midsummer were also included: *Sphagnum magellanicum* from near Ottawa, Canada⁴ and *Philonotis fontana* from New Hampshire⁵. Eleven species of aquatic macrophytes were included: data from Boyd⁶ on eight genera (*Elodea*, *Myriophyllum*, *Vallisneria*, *Potamogeton*, *Lemna*, *Paspalum*, *Najas*

and *Phragmites*) and on three genera from the south-eastern United States (*Eichhornia*⁷, *Justicia*^{7,8} and *Pistia*⁹).

Concentrations of elements were obtained from leaves of vascular species. There were eight woody species from Brookhaven, New York (*Quercus coccinea*, *Q. alba*, *Q. ilicifolia*, *Pinus rigida*, *Gaylussacia baccata*, *Vaccinium vacillans*, *V. angustifolium* and *Kalmia angustifolia*)¹⁰, two woody species from central Oklahoma (*Quercus stellata* and *Q. marilandica*)¹¹ and six species from New Hampshire (*Acer saccharum*, *A. spicatum*, *Betula alleghaniensis*, *Fagus grandifolia*, *Picea rubens*, *Prunus pensylvanica*)⁵. Data on four herbaceous species from the south-eastern United States were also included: *Saururus cernuus*¹², *Juncus effusus*¹³, *Typha latifolia*¹⁴ and *Alternanthera philoxeroides*^{7,8}. Finally, there were unpublished data for woody and herbaceous species from South Carolina: *Alnus serrulata*, *Myrica cerifera*, *Salix nigra*, *Scirpus cyperinus*, *Erianthus* sp., *Polygonum punctatum* and *Sagittaria latifolia*.

Phosphorus and nitrogen concentrations were correlated significantly across the species examined ($P < 0.001$, Fig. 1). Within the three broad categories of plants, correlation between P and N in vascular and non-vascular plants was significant ($r = 0.90$ and 0.85 respectively, $P < 0.001$). P and N were not correlated significantly in the aquatic macrophytes ($r = 0.46$, $P > 0.05$). When P concentrations were regressed against N concentrations, the slopes of the regression lines were similar for all three categories. The regression coefficients ($b \pm 1$ s.d.) were as follows: non-vascular plants, 0.095 ± 0.016 ; aquatic macrophytes, 0.105 ± 0.071 , and vascular plants, 0.108 ± 0.010 . The intercept value in each of these regressions and in the overall regression across species was zero.

Ca and Mg concentrations were correlated significantly in non-vascular plants ($r = 0.65$, $P < 0.01$), aquatic macrophytes ($r = 0.78$, $P < 0.01$) and vascular plants ($r = 0.48$, $P < 0.05$) and across all species ($P < 0.001$, Fig. 2). When Mg concentrations were regressed against those of Ca, the slope values ($b \pm 1$ s.d.) were 0.089 ± 0.029 , 0.124 ± 0.033 and 0.143 ± 0.051 for non-vascular plants, aquatic macrophytes and vascular plants, respectively. A correlation matrix among N, P, K, Ca and Mg concentrations across all species was calculated. Correlations between other element pairs were not statistically significant ($P > 0.05$), except for a correlation between Mg and K ($r = 0.50$, $P < 0.001$).

Correlations between pairs of elements have been reported

Fig. 1 Relationship between concentrations (% dry weight) of P and N across 54 species. Only leaf concentrations were used from vascular plants. Correlation is significant at the $P < 0.001$ level. $r = 0.84$, $b \pm 1$ s.d. = 0.10 ± 0.009 . ●, Vascular; □, aquatic macrophytes; △, non-vascular.

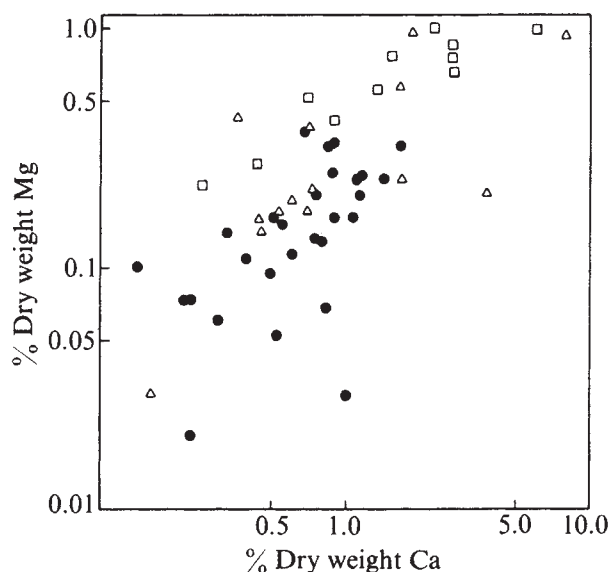
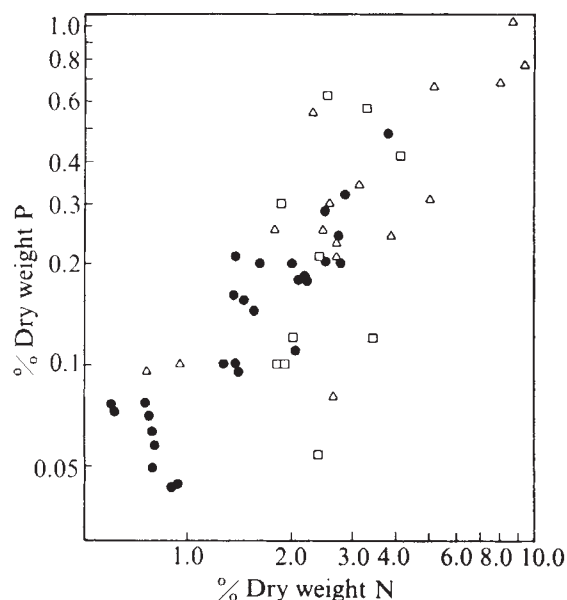


Fig. 2 Relationship between concentrations (% dry weight) of Ca and Mg across 53 species. Only leaf concentrations were used for vascular plants. Correlation is significant at the $P < 0.001$ level. $r = 0.70$; $b \pm 1$ s.d. = 0.13 ± 0.019 . Symbols as for Fig. 1.

before in laboratory studies but not for natural vegetation. In ryegrass, *Lolium perenne*, grown in nutrient solutions containing various concentrations of sulphate and nitrate, a relationship was observed between gram atoms of protein S and protein N (ref. 15). Both S and N are important constituents of proteins and amino acids. Similarly, N and P are closely associated in both cytoplasmic and nuclear material, including cell membranes, nucleic acids and ATP, ADP and NADP. Correlations between N and P reflect their close association in plant biochemistry, particularly protein synthesis. The lack of correlation between N and P in aquatic macrophytes may be due to the narrow range of N values reported here, or to other factors such as "luxury consumption" of N when environmental concentrations of P are deficient. A relatively constant ratio of P to N (1:10) across species and types of plant suggests a very general association between these elements.

Calcium and magnesium have structural and enzymatic functions in the plant cell. Mg is central to the structure of chlorophyll, and Ca is a component of pectins in the cell wall. The correlations between Ca and Mg concentrations is probably a consequence of the close association between these elements in metabolism and photosynthesis¹⁶: both elements are enzyme activators in metabolic reactions. As chloroplasts may contain 50% or more of the total leaf Mg and 60% of the total leaf Ca¹⁷, a correlation between the concentrations of Mg and Ca across species is to be expected. The ratio of Mg:Ca appears to be more variable than that of P:N across different plant types. Over all species, the Mg:Ca ratio was 1:7.7. Mg and K, which were also correlated, are essential for certain enzyme activities in all plants and both are linked to photosynthesis¹⁸. Furthermore, concentrations of these elements could be correlated because of similar chemical properties. N and P share common valence states, as do Ca and Mg. Their mechanisms of uptake across cell membranes are probably similar.

There have been previous attempts to find P:N and Mg:Ca ratios that maximise crop yields in agricultural plants¹⁸ but little work has been done with natural species. Gerloff and Krombholz¹⁹ found that the critical contents of N and P—the minimum contents in plant tissue associated with maximum growth—for several species of aquatic angiosperms were 1.3% and 0.13% respectively. For spruce and pine seedlings, the critical concentration of N is approximately 1%, and that for P is 0.08–0.1% (refs. 20 and 21). The critical P:N ratio seems to be 0.1, which is the same ratio I have found

using many more species. The evolution of constant ratios of elements may have been related to the maintenance of biochemical equilibria within cells that maximise protein synthesis and tissue production in natural environments.

Undoubtedly, concentrations of elements in the environment, as well as a combination of edaphic or biological factors which result in luxury consumption or nutrient deficiencies of elements in leaves, can produce a correlation or lack of correlation of concentrations in plants. But in view of the number of species and variety of sites included in this analysis, similarity in the biochemical function of pairs or groups of elements seems to be the most important factor contributing to the observed correlations.

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Adjusting to the changes to and from Daylight Saving Time

A GREAT deal of attention has recently been paid to the circadian rhythms that have been found to exist in a number of human physiological and behavioural processes. There are several ways in which such rhythms can be disrupted, and extensive studies have been made of the effects of prolonged isolation¹, abnormal working hours², and flight across several time zones³. The adoption of Daylight Saving Time (DST) is another possible cause of disruption, but seems to have been generally ignored in spite of the large number of people potentially affected. We have found that significant disruptions of behaviour occur during adaptation to the time change.

More than 25 countries around the world now use a DST system of one kind or another. The system used in Britain comprises a notional shift in time zone, eastward (that is "losing" 1 h) in the spring and westward (that is "gaining" 1 h) in the autumn. Although the general effect of DST on energy consumption and behaviour patterns has been studied quite extensively⁴, we can find no studies reporting the disruptions in behaviour that might be produced by the actual time changes themselves.

The high cost of "jet lag" studies usually requires that fairly large shifts in time zone are considered, since comparatively few subjects can be used, and there tend to be large

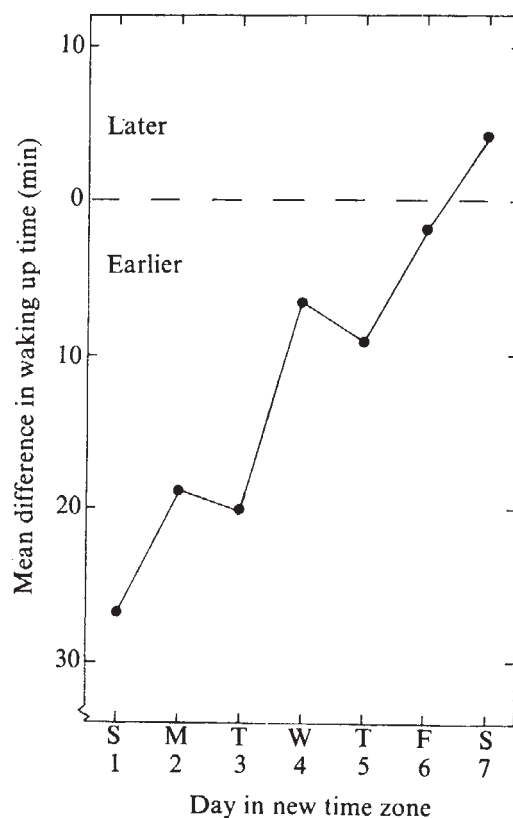
individual differences. Time zone shifts of less than 3 h have thus typically been ignored. In the changes to and from DST, the investigator is presented with a whole population that is trying to adapt to a "new" time zone. We therefore considered the changes to and from DST to be ideal opportunities for the study of adaptation to small shifts in time zone using a relatively large number of subjects.

The study was conducted during the change back from British Summer Time (BST) to Greenwich Mean Time (GMT) in the autumn of 1974. At 0200 GMT on Sunday October 27, the clocks were officially moved back 1 h (that is from 0300 BST to 0200 GMT). Sixty-five subjects (55 of them female) living in the Brighton area took part in the study. For 6 d before the DST change and 11 d after it, the subjects were asked to record their time of waking; and at 0900 (clock time), to measure their oral temperature (by a 3-min sublingual insertion of a standard clinical thermometer) and rate their feelings of alertness on a scale from 0 to 20.

The process of adaptation to the time change was most apparent in the mean waking up times. Since an hour was effectively "gained" by the change, earlier waking up times than normal were expected. When the waking up times of the days following the DST change were compared with those of a normal week a clear disruptive effect was observed (Fig. 1). This effect was still statistically significant on the fifth day in the "new time zone" ($t = 1.75$, d.f. = 38, $P < 0.05$). The subjective alertness scores at 0900 were, as expected, positively correlated with the length of time awake ($r = +0.8$, d.f. = 15, $P < 0.01$), and there was a corresponding slight increase in the ratings of perceived alertness during the week following the DST change.

The circadian rhythm in oral temperature normally produces a rise of about 0.1 °C from 0900 to 1000 (ref. 5). Since, after

Fig. 1 The mean difference in waking up time between a day of the week following the DST change and the corresponding day of a week unaffected by the change. Each point represents the mean difference of the 39 subjects who returned waking up times for all 17 days of the study (a similar curve was produced when all 65 subjects were included.)



the time change, the readings were actually taken at 1000 as defined by the "old" time zone, any lack of adaptation in the rhythm would be indicated by a corresponding rise in temperature. Unfortunately there were marked fluctuations in the air temperature at Brighton over the 17 d and the mean oral temperature of the 65 subjects was found to be significantly correlated with these fluctuations ($r = +0.7$, d.f. = 15, $P < 0.01$). The presence of such correlations and other uncontrolled factors, such as the menstrual cycle of the female subjects, makes interpretation of the oral temperature data rather difficult. Nevertheless, when the effect of air temperature was partialled out using a linear approximation, a rise in adjusted oral temperature was found in the 6 d following the DST change.

The process of adaptation in waking up times took much longer than the one day (or less) that would be predicted from studies of the "jet lag" effect⁶. A possible reason for the discrepancy is that in travel across time zones both the physical cues such as sunrise and sunset and the social cues such as working, eating and sleeping habits, change in a way that encourages adaptation to the new cycle. In the DST change, however, only the social cues change, the physical ones remaining synchronised to the "old" cycle. While much of the recent work has emphasised the importance of social cues over physical ones, it is possible that the latter may have impeded the process of adaptation.

This study was concerned with a westward (autumn) shift in time zone. Studies³ of the jet-lag effect have suggested that eastward shifts are the more disruptive and an even greater disruption might thus be expected in the DST change that occurs in the spring. Since "jet lag" has been shown to impair performance³, it is possible that the disruption induced by DST changes (particularly in the spring) may have a similar effect. A preliminary study was thus undertaken to determine whether this disruption was sufficient to be associated with a measurable increase in road accidents. Evidence of an increase during the week following the spring DST change was found, but further validation of this result is clearly required.

In conclusion, it seems that adjustment to the time changes associated with DST is not instantaneous, and that significant disruptions in behaviour may occur during adaptation to the new cycle. The presence of such disruptive effects following a 1-h transition reinforces the importance of studying shifts in time zone, even when the time change involved is considerably less than that typically encountered in transcontinental flight.

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to be close to, and ideally higher than, the fusion frequency for the human visual system. With the display luminances generally encountered in normal broadcast television, field frequency flicker is negligible in countries using 60 fields s^{-1} , and, though often noticeable, has proved acceptable in those using 50 fields s^{-1} .

It was somewhat surprising, therefore, while investigating the effect of line and field scanning directions on the legibility of alphanumeric television displays with luminances which were higher than normal, to find that the flicker was more visible when the field was scanned from left to right (L-R), right to left (R-L) or bottom to top (B-T) than it was for the normal top to bottom (T-B) direction. A closer scrutiny seemed to indicate that the increased visibility was due to greater sensitivity to the movement of the luminous emittance through the display in these directions of field scan. No previous report of this phenomenon is known to the authors, and the following subjective experiment was carried out to verify its existence in quantitative terms.

A vidicon camera channel, operating at 625 lines per picture and 50 fields s^{-1} , and used with a slide projector, provided a source of still pictures. A facility for reversing the direction of field scan was a standard feature. A 350-mm television picture monitor was modified to allow the direction of field scan to be switched in synchrony with the picture source so that the displayed picture always appeared the right way up. A 500-mm square matt-grey mask with a 210-mm square cut-out was positioned in front of the monitor. The 1:1 aspect ratio allowed field scans (L-R) and (R-L) to be produced, with the displayed picture dimensions unchanged, by turning both the slide and the monitor (behind the mask) through 90°.

The average illuminance of the specially equipped viewing room, measured at table height, was 50 lx. Gathered white curtains, subtending a horizontal angle of about 120° at the observer's eyes, were used as a backcloth, and concealed lighting was adjusted to give a backcloth luminance of 3.5 $cd\ m^{-2}$ over a large area behind the monitor. The luminance of the grey mask surround was 1.6 $cd\ m^{-2}$.

Four pictures were chosen from a large available stock to exhibit from well above average to about average degrees of flicker. They comprised two head-and-shoulders portraits, one scene and one black line drawing on a white background. The highlight luminance of the display monitor was set to 200 $cd\ m^{-2}$, which, in conjunction with the luminance of the inactive screen, 1 $cd\ m^{-2}$, defined the maximum available contrast ratio of 200:1.

The observers were colleagues working in the fields of television and photography. Pairs of observers attended each test session, and were seated at a table in front of the monitor at a viewing distance of 1.26 m, six times the picture height. The centre of the screen was at a height of 1.05 m and was therefore a little below eye level for the average seated observer.

Initially, two tests were planned—one in which the (T-B) and (B-T) directions of field scan would be present, and the other for the (L-R) and (R-L) directions. To the extent that interlace might affect the visibility of flicker, the presence or absence of interlace was included as an additional variable, the non-interlace scanning standard being 312 lines per picture, 50 pictures s^{-1} . A balanced test design ensured that each picture was associated with each combination of scan direction and interlace condition, and replication gave a total of 32 presentations per viewing session. Where possible, the same 24 observers attended each test, and each of the 12 viewing sessions in each test lasted on average 20 min. The observers were asked to assess the flicker using the following 5-grade scale: imperceptible; perceptible, but not annoying; slightly annoying; annoying; very annoying.

To eliminate the remote possibility that the variation of visibility with field scan direction was an equipment effect

Visibility of flicker in television pictures

A PRIME requirement in a television system is that the flicker inherently associated with the displayed picture should be minimal. The visibility of such flicker is generally considered to depend primarily on the picture luminance, the angle subtended by the picture and the field repetition frequency. The field frequency should therefore be chosen

and not a visual perception effect, a third test was carried out with (T-B) and (B-T) scan directions as in test 1, but with both the display monitor and the slides turned upside down. Any contribution to the flicker caused by the equipment would thus move through the pictures in opposite directions in tests 1 and 3. Also, a check made by a small number of observers with the room lights turned off confirmed that no significant flicker effects were being caused by the interaction of the display field frequency and the room illuminance frequency.

Results are quoted in terms of the mean opinion score, obtained by allocating the values 1, 0.75, 0.5, 0.25 and 0 to the grades "imperceptible" through to "very annoying" and calculating the mean value for each picture condition. An analysis of the sources of variance in the results for the individual pictures in all three tests indicated that the interlace and the replication factors did not contribute significantly to the total variance. Thus, in all three tests, average values of mean score were obtained for each picture/scan direction combination. These averaged values in tests 1 and 3 showed no significant or systematic differences and could be further averaged, indicating that the phenomenon being investigated was truly of a visual perception nature. For similar reasons, the results for (L-R) and (R-L) direc-

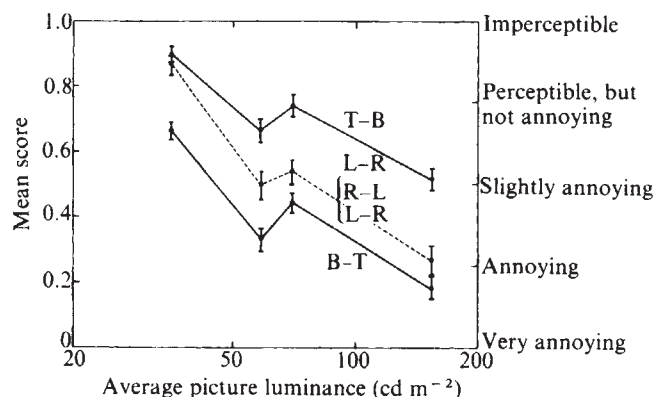


Fig. 1 Visibility of flicker for the various directions of field scan. —, Average of tests 1 and 3; ---, test 2.

tions of scan in test 2 were averaged. The average mean scores are plotted as functions of the average picture luminance in Fig. 1. The kink in each curve indicates that average luminance is not the most appropriate objective measure of susceptibility to flicker, but it is easy to define and measure. Furthermore, the main interest is in the relative vertical displacements of the curves and the horizontal scale is mainly a convenience to separate the effects on the different pictures. The confidence interval associated with each data point represents plus and minus one standard error in the mean value, and the differences between the data points for the normal (T-B) scan direction and the corresponding data points for the other scan directions are all highly significant ($P < 0.001$) except for one case, easily identified in Fig. 1, where the difference is not significant.

A fuller investigation and explanation of this phenomenon must be left to those working full time in the field of human visual perception. One possible explanation is that, because of television viewing experience, people have adapted to 50-Hz flicker with the field scanned from top to bottom. Such adaptation would have to be a more powerful and long term effect than that usually associated with continuous movement in one direction; after a few minutes exposure to upwards movement, the associated flicker was still more visible than that apparent the instant the movement was switched to its normal downwards direction.

The existence of such a long term adaptation effect might be an additional reason why visitors from countries with

60 fields s^{-1} television, who have not adapted to 50-Hz flicker, apparently find it so much more annoying. Indeed, one of the reasons for describing the experimental arrangements in some detail is the hope that the experiment may be replicated, still using 50 fields s^{-1} television, in a country where the normal television standard is 60 fields s^{-1} or, preferably, where no television service exists. The results of such an experiment might indicate whether the phenomenon is acquired, which seems more likely, or is inherent. A qualitative check with 60 fields s^{-1} television indicated that the movement associated with the flicker was again more visible in the upwards direction. The magnitude of the increase seemed to be smaller than for 50 fields s^{-1} television, but its existence suggests that any adaptation effect is not precisely linked to velocity.

Meanwhile, confirmation of the existence of the effect suggests that the normal (T-B) direction of field scan should be preferred for new visual telecommunications systems, particularly where a 50 fields s^{-1} standard and high display luminance are envisaged.

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Differing emotional response from right and left hemispheres

To study what might be called the 'emotional vision' of the right and left hemispheres of the human brain, a specially designed contact lens was used to show cine films to the right or left hemisphere only. Films were chosen to provoke different kinds of emotional response. We wished to find whether the two hemispheres would take and utilise the stimulus of the film to generate a different emotional response despite the common input. In recent years clinical and experimental data have supported the hypothesis that different emotional reactions follow damage to the right or the left hemisphere¹⁻⁵. We report here the first part of an investigation in which films were perceived by different hemispheres in normal subjects and the subjects were then asked to judge and rate the films. There is of course no intention of suggesting that information implanted at one hemisphere cannot be transferred to the other^{6,7}; nevertheless, the basis of this research is that the two hemispheres can differ in their vision of the world and that each in some respects formulates its own separate and distinct emotional vision of what it sees. We seek to answer the question of what visual experience is like when that which the subject sees enters his nervous system on only one half of his brain, but this is part of the much wider question of how the two hemispheres of the brain see the world and of the relationship between the two visual halves of the brain.

As a preliminary to the experiment reported here a pilot study was conducted in which seven different films were shown to 14 subjects using free vision to assess the nature of their response. Each film was rated on the four dimensions of humorous, pleasant, unpleasant and horrific, using a scale marked 1-9. On the basis of the ratings given, three films were selected for subsequent use. These were a Tom and Jerry cartoon film (duration 3.20 min), a medical film depicting a surgical operation (3.30 min) and a travel film of Lucerne (3.20 min). The sound tracks were not used.

The volunteer subjects were students aged between 18 and 24. All were normally contact lens wearers. They were all right-handed as judged by the Annett handedness questionnaire. Each of the 21 subjects was assigned alternatively to either the left or right visual field. The films were shown to one hemi-

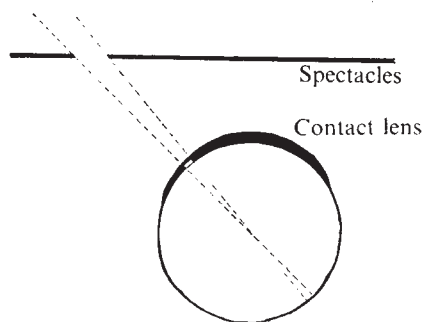


Fig. 1 The optical system.

sphere only for each subject. The order in which the film clips were shown was balanced according to a Latin square design.

The lenses were first inserted and checked for accuracy of fitting. The subject's own spectacles were worn to provide for corrected vision. These were blacked out as we shall describe. Light sensors were fitted to the ear to record heart rate and electrodes to the fingers to record electrodermal response. The results obtained from these measures will be reported on a separate occasion. A check was made that the subject could see the whole area of the screen. The subject was then instructed to relax and watch the film. At the conclusion of each film the subject was asked to rate it on a scale of 1-9 on the four dimensions of humorous, pleasant, horrific or unpleasant. This was done manually on the form provided, the subject continuing to wear the contact lenses meanwhile.

The lenses were constructed of a rigid plastic. They were plano, and of the following dimensions: back optic curve—8 mm: 8.5 mm: 10.5 mm: diameter—7 mm: 7.5 mm: oval diameter—10.0 mm. The lenses were opaque except for a 1-mm wide vertical slit decentred 2 mm from the optic axis. For left hemisphere vision the slit was decentred 2 mm to the right, for right hemisphere vision 2 mm to the left. Both lenses were truncated 1.5 mm. The truncated edge of the lens rested on the lower lid of the eye which prevented the lens from rotating, thus maintaining the vertical slit in a stable position throughout the experiment.

Vision was binocular throughout, the subjects wearing lenses in both eyes. The subjects wore their own spectacles in conjunction with the contact lens during the tests. The corresponding lens of the spectacles was covered with black insulating tape to leave a clear slit corresponding to that of the lens and on the same side. This was done to provide what we at that time regarded as an additional safeguard against stray light entering the eye from an undesirable angle (Fig. 1).

The results are presented in Table 1. This gives the mean

Table 1 Response to film viewing on the right hemisphere (RH), the left hemisphere (LH) or in free vision (control)

	Tom and Jerry	Travel	Medical	Total
Humorous				
RH	6.00	1.80	1.80	9.60
LH	6.27	1.27	1.00	8.54
Control	6.57	1.50	1.00	(9.07)
Unpleasant				
RH	2.80	3.50	6.00	12.30
LH	1.45	1.45	5.45	8.35
Control	1.42	1.07	6.85	(9.34)
Horrific				
RH	2.50	1.50	5.00	9.00
LH	1.54	1.36	3.54	6.44
Control	1.71	1.07	3.85	(6.63)
Pleasant				
RH	6.40	6.20	2.30	14.90
LH	6.72	5.81	2.54	15.07
Control	6.14	6.92	2.07	(15.13)

judgment for each film on the 1-9 scale. Ratings for films viewed on the right hemisphere are compared with those viewed on the left hemisphere. These in turn are compared with the results of the pilot study in which the films were shown in free vision to 14 subjects and rated according to the same criteria.

A Friedman two-way analysis of variance performed on the data shows that there are significant differences between the left hemisphere, right hemisphere and control conditions ($\chi^2 = 32.91$; $P < 0.001$). When comparison was made between the right and left hemisphere significant differences were found for the judgment of unpleasant for all films, the right hemisphere judgment as more unpleasant than the left (Tom and Jerry, $U = 71$, $P < 0.01$ level; Travel, $U = 73$, $P < 0.01$; Medical, $U = 53$, $P < 0.01$, Mann-Whitney U test). Significant differences were also found for the horrific category (Tom and Jerry, $U = 77$, $P < 0.01$; Medical, $U = 51$, $P < 0.01$, Mann-Whitney U test). Significant differences in this category were not found for the travel film.

No significant differences were to be observed for the categories humorous or pleasant. The control did not differ significantly from the results for the left hemisphere.

We report here also the results of a study in which we obtained perimetric visual field data from subjects wearing the lenses. This was an experiment conducted on twelve subjects in which 'neuroticism' and 'extraversion' were studied in an attempt to find differences between the response on one hemisphere and that on the other. The Eysenck Personality Inventory was divided into alternate types of questions. These were printed out in large lettering so that they were easily visible, half were given to the left hemisphere and half to the right. The results of this experiment proved to be negative but more importantly we collected visual field data from each of the subjects. This was obtained in a darkened room using a standard perimeter on maximum size and illumination setting.

The subjects wore the contact lenses and their spectacles as previously described. The subjects placed their chin on the rest and were instructed to look straight ahead. The subject was asked to say "yes" when he saw the light appear and "no" when it disappeared. The direction and angle by which the light spot was brought into the subject's field of vision was randomised. The visual fields so obtained are shown in Fig. 2. It can be seen that vision is restricted to a small oval area occupying only a fraction of the visual field, located some 20° either to the left or right of central vision.

One striking incidental observation followed from this investigation after subjects removed the lenses at the conclusion of the experiment while still sitting at the perimeter apparatus. They were greatly surprised to find that they had not been stimulated at the centre of their vision. To them what they saw appears as part of subjective space undeniably central and certainly not as something displaced from the midline.

We report therefore a new phenomenon of 'centring' in vision, that is, the taking of small parts of vision displaced to one side and creating of it a realignment in experience such that it comes to occupy the centre of the experienced visual world. The brain not only takes a small restricted input and uses it to fill the whole span of visual experience, but so also does it take islands of vision displaced quite far to the periphery and make of them the centre point of the experienced visual world. The brain, in other words, creates its own centre of vision in spite of the lateral displacement of its image.

We suggest on the basis of these results that the two hemispheres of the brain of man possess an essentially different emotional vision of the world. The objective world although physically the same for each side, has a different perspective imparted to it. We suggest that the right hemisphere adds its own emotional dimension which represents the thing perceived as more unpleasant and horrible and thus aligns itself more with the characteristic perception of the depressive patient than with that of the normal individual. Whatever the nature of this form of perception, we believe that each hemisphere has its own

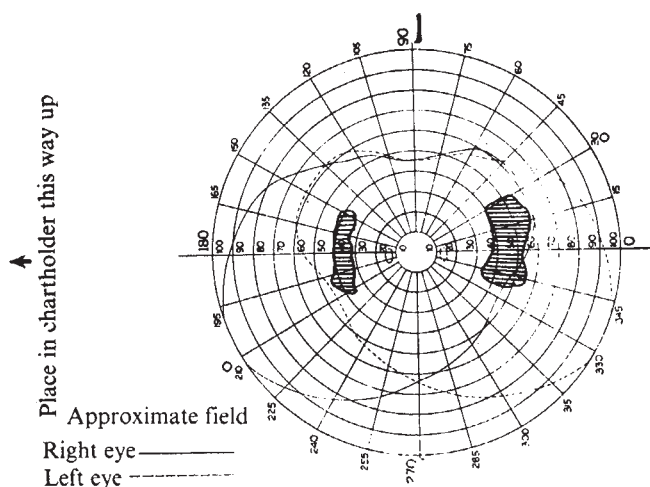


Fig. 2 Areas of vision for a typical subject wearing lenses for the right or left visual field.

distinct emotional vision of the world and that each makes a unique contribution to the whole. At the same time the left hemisphere scores so closely resembled those for the free vision condition where it may be assumed that both hemispheres are stimulated, to suggest that it is usually the left hemisphere perception which predominates. The extra dimension of the emotional focus of the right hemisphere seems to be suppressed in ordinary vision. How far the right hemisphere enters into the everyday emotional appraisal of the environment is a matter for debate. With regard to the question of the ultimate unity of emotional judgment our results suggest that the left takes precedence over the right but we cannot discount the possibility that the emotional vision of the right is used in ways which cannot as yet be specified.

There could be a parallel to ideas of unconscious determination of conduct in the view that the right hemisphere provides a source for unconscious motivation within the brain. The right hemisphere does not seem to be denied access to consciousness, however, but instead contributes an alternative voice to mental action at the conscious level.

It may be that the differences reported here are cognitive ones arrived at as the result of viewing the film through one hemisphere channel rather than another. It is hoped that our records of heart rate and electrodermal response recorded simultaneously while viewing will allow us to distinguish whether some fundamental difference of emotional functioning between the hemispheres is indicated, or whether these results can be explained on the basis of a cognitive assessment made during or after viewing has taken place.

In the initial report on the research using this technique⁶ we suggested that lateralisation had been successfully achieved although we could not discount the possibility that some input may pass to the midline or beyond to the opposite hemifield. On the basis of the lateral differences observed in that study and the visual field data presented here we now feel more confident that the hemifield lateralisation has been successful and we believe the technique to be a particularly useful one in cerebral laterality research.

We also believe that these techniques will be important for the restriction of input to small parts of the visual field to study functions related to local parts of the retina.

We conclude therefore that there exists a different emotional vision between one hemisphere and the other in the normal human and that the right hemisphere has an emotional vision which is usually suppressed but is characterised as more unpleasant and horrific than is that of the left.

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Significance of two frequency bands in long distance vocal communication in the green treefrog

VOCAL communication is crucial in the reproductive biology of the green treefrog (*Hyla cinerea*). The male calls at night near a suitable breeding site: the acoustic signal serves for both localisation and species recognition by the female^{1–3}. A typical mating call has two frequency bands of about the same relative amplitude: the lower one consists of one component of about 1 kHz; the upper one consists of one to four (usually two) components at about 2.7–3.3 kHz (ref. 2). Both frequency bands are pertinent for species discrimination at a moderate sound pressure level (SPL), corresponding to the SPL at 2–4 m from vocalising males^{4,5}. This report discusses the significance of the two frequency bands in communication over greater distances. Not only does the SPL decrease with distance, but also the relative amplitudes of the two spectral peaks change because excess attenuation is frequency dependent. In general, high frequencies attenuate more rapidly than do low frequencies^{6,7}. I found that a low frequency component of the call was more attractive to females than were high frequency components. Furthermore, in discrimination experiments females detected attenuation of a 3-kHz component relative to a 0.9-kHz component at moderate to high SPL but failed to do so at low SPL.

The minimum SPL necessary to attract gravid female treefrogs within 5 min from 1 m in front of a Nagra DH speaker is here defined as a behavioural threshold. Three kinds of tone bursts were presented: 0.9, 3.0 and 2.7+3.0 kHz (the two components had the same relative amplitude and were phaselocked). The sounds were recorded and played back with a Nagra S tape recorder. A Brüel and Kjaer 2209 sound level meter was used to adjust SPL (dB relative to 2×10^{-4} μ bar r.m.s. 'fast'). Tests were carried out at night outdoors on a level cement driveway at air temperatures of 20–26 °C. Each of 34 females was collected in amplexus near Savannah, Georgia and tested individually at 1–4 SPL per stimulus (about 2 min between trials). Nine of the animals responded to two different sounds. The females had lower behavioural thresholds to the low fre-

Table 1 Responses of 34* female *H. cinerea* to synthetic calls of variable sound pressure level

Stimulus	Number tested	SPL (dB re 2×10^{-4} μ bar)	Number of trials	Oriented to speaker	Approached speaker (within 50 cm)	Touched speaker
3.0 kHz	12	95	10	3	3	2
		90	11	2	0	0
2.7+3.0 kHz	12	77	8	6	5	5
		72	12	7	5	4
0.9 kHz	19	60	13	11	9	4
		55	9	5	2	0

*Nine females responded to two different sounds. Additional responses were observed at higher SPL than those included in the table.

Lowest SPL which attracted females is considered to be the behavioural threshold for the stimulus used. Females failed to respond when the SPL was lowered by 5 dB. Only two of the four females which touched the speaker when 2.7+3.0 kHz was broadcast at 72 dB were tested at 67 dB. Call duration was 0.16 s and repetition rate was 0.8 s. See Gerhardt⁴ for details of the synthesis of experimental stimuli.

quency call (0.9 kHz) than to the two-component (2.7+3.0 kHz) high frequency call (Table 1). The temporal patterning (300 s^{-1} beating) of the latter stimulus undoubtedly contributed to its lower threshold (Table 1) as compared with the 3-kHz stimulus*. Because of relatively high background noise levels, I consider these threshold determinations to be crude and conservative; nevertheless, I emphasise the significant differences in the relative attractiveness of the three sounds.

Neurophysiological studies of *H. cinerea* from Savannah are consistent with the above behavioural results. Moffat and Capranica⁹ found two (of three) populations of neurones in the auditory nerve that seem to be directly involved in mating call processing: in one, neurones are most sensitive to frequencies at about 0.6–1.2 kHz; in the other, neurones are tuned to about 3.2–3.6 kHz. The low frequency units are, on the whole, much more sensitive than the high frequency units. Lombard and Straughan¹⁰ obtained comparable results when they recorded evoked potentials from the midbrain of *H. cinerea* from Florida.

Besides indicating that low frequency components of the call will attract the female from a greater distance than high frequency components, the above results suggested that in call (species) recognition the relative importance of the two spectral peaks may depend on the SPL of the sounds. Accordingly, 78 females from Savannah were tested in dis-

crimination experiments. Each animal was released midway between two Nagra speakers placed 4 m apart. Synthetic calls consisting of two phase-locked components of 0.9 and 3.0 kHz having the same relative amplitude were broadcast from one speaker; and calls with the same components having unequal relative amplitudes from the other. Calls were presented alternately every 0.4 s. I equalised the SPLs at the point where I released each female, and defined a response as a physical contact with a speaker. At a low playback level (65 dB) females discriminated against a synthetic call having an attenuated lower peak but failed to discriminate against a call with an attenuated upper peak (Table 2). At a high SPL (85 dB) females discriminated against the call with the attenuated upper peak but failed to discriminate against the call with attenuated lower peak. Note that 85 dB is above the behavioural threshold for a high frequency call with two components (2.7+3.0 kHz) but below the threshold for the 3-kHz call (Table 1). Because fine temporal as well as spectral patterns are pertinent, generalisation of the above discrimination results using synthetic calls with two high frequency components (0.9+2.7+3.0 kHz) will be important.

In certain uniform conditions, distance attenuation from a point source increases monotonically with frequency¹¹. Direct measurement by Morton⁷, however, showed that in complex, natural environments frequency-dependent excess attenuation differs dramatically from habitat to habitat. Preliminary analyses (H.C.G., unpublished) of spectral changes in natural and synthetic frog calls after propagation through natural environments corroborated Morton's results. Furthermore, I found that the elevations of both the sound source and measuring point had a significant bearing on the attenuation of the high frequency relative to the low frequency components.

I suggest, therefore, that the low frequency components of the male's call attract the female at a distance. As she approaches the male (or chorus), high frequency components also become important for species recognition. The distance at which the high frequency components have sufficient energy to affect the female's behaviour will depend on the habitat and locations of the frogs.

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Table 2 Responses of 78* female *H. cinerea* in discrimination experiments

SPL (dB)	Number of females responding to: 0.9 kHz (0 dB) + 3.0 kHz (0 dB)†	Number of females responding to: 0.9 kHz (–12 dB) + 3.0 kHz (0 dB)‡	Probability (Two-tailed binomial)
65†	13	1	0.002
70–75†	11	1	0.006
85+	14	7	Not significant
	0.9 kHz (0 dB) + 3.0 kHz (0 dB)	0.9 kHz (0 dB) + 3.0 kHz (–9 dB)	(Two-tailed binomial)
65	6	6	Not significant
70–75	8	2	0.080
85	9	0	0.004

Each female was used in only one test, and only first responses were tabulated. Duration of synthetic calls was 0.16 s and repetition rate was 0.8 s. See Gerhardt⁴ for further details.

*Five of these females were used in threshold trials (Table 1) after they responded in a discrimination test.

†The highest modal SPL of the mating calls of *H. cinerea* measured in the field was 93 dB (RMS) at 0.5 m (ref. 5). If this is taken as a reference point and it is assumed that attenuation is solely due to spherical spreading, then these levels (60, 70–75 and 85 dB) correspond to distances of about 13, 7–4, and 1.3 m respectively from the sound source.

‡Relative amplitudes were measured (General Radio 1900-A wave analyser) from recordings (Nagra 4.2 recorder, Sennheiser 815 microphone) made 2 m from the speakers on a cement substrate.

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Rates of size increase and of phyletic evolution

THE RATE of phyletic evolution (evolution of the non-branching, non-cladogenetic type) is highly correlated inversely with the overall population size of genera and species. Factors which can materially decrease overall population size include increases in one or more of the following: number of biogeographic entities; number of communities; degree of climatic differentiation; and degree of marine regression on the continents (smaller populations of shallow water, marine organisms with regression). When all of these factors act in one direction rates of phyletic evolution are high or low; when they interfere with each other rates are intermediate^{1,2}.

An additional factor is size. Cope's Rule long ago formalised common knowledge that during evolutionary history many taxa tend to increase in size. Other taxa tend to decrease in size with time. Hallam's data³, based on Jurassic bivalves and ammonites, indicates that genera and species showing an evolutionary (not environmentally) large rate of size increase have shorter time ranges than do taxa showing a small rate of size increase or no increase. Hallam's data, as well as those available from other time intervals, indicate that such size changes are nonlinear over long time intervals. Hallam³ has used Fürsich's data to show that larger Jurassic fossil taxa from one region are less numerous as individuals than is the case with smaller fossil taxa. This last relationship is characteristic of living animals (see Reish⁴ for a typical example).

Stanley⁵ points out that most species of higher taxa (such as Class, Order, or Superfamily) are in the lower part of the total size range known for the higher taxon.

Figure 1 indicates the upper and lower size limits imposed on the species of higher taxa by their physiological, mechanical and ecological attributes as discussed by Stanley. He gives ecological and physiological explanations of why most species belonging to a higher taxon will tend to the lower rather than the upper size limit. Figure 1 indicates that many species will evolve phyletically with no change in size, whereas others will increase or decrease. Of the species changing their size, some will do so rapidly and others slowly. Stanley's Fig. 3 makes it clear that size increases will tend to affect a relatively small number of species belonging to any higher taxon.

Hallam's 'curves' (from his Fig. 2) for change in size of Jurassic molluscs with time can be interpreted as different segments of the curves of size change against time (Fig. 1) for forms undergoing rapid, slow or fluctuating changes in size. Consideration of Fig. 1 suggests that Hallam studied too small a sample through geological time to have been able to see more than a small, albeit significant, portion of the total size change curve for any taxon he considered.

The fossil record implies that most phyletic evolutionary changes in size have been moderate, since increasingly smaller percentages of species approach the upper and lower size limits known for each major group.

Consideration of sizes and size changes through time for fossil and recent organisms makes it reasonable to conclude that the various rates of size change are about equally likely for both increases and decreases. The data also suggest that lower rates of increase and decrease are more common than are higher rates.

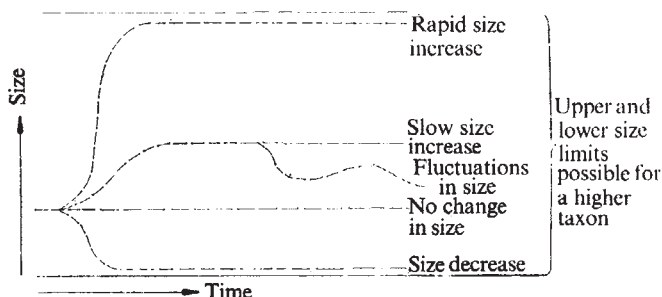
Hallam also observed that "... younger taxa are often appreciably smaller than their evolutionary precursors, but

with no discernible size transition from one to the other." This conclusion follows (Fig. 1) because the modal size for any animal group is far closer to the lower than to the upper size limit characteristic of the group. The chances of sampling adequately the fossil record for the brief, downward size changes is less than for the more extended, upward size changes—with both changes occurring at the same rate. Consideration of the Silurian-Devonian brachiopod record accords with Hallam's observations about the absence of intergrades showing size decrease as opposed to the more extensive intergrading sample found for size increase.

Hallam's data³, as well as Silurian-Devonian brachiopod data, strongly indicate that evolutionary changes in size occur at non-uniform rates. Hallam's observations are founded on intraspecific and intrageneric evolution in Jurassic molluscs whereas mine are based on intrageneric and intergeneric relations among Silurian-Devonian brachiopods. Those groups of Silurian-Devonian brachiopods showing a significant size increase with time exhibit a nonlinear rate of increase. The rate of increase in size is a function of the magnitude of the increase, as also suggested by Hallam's data. For example, during the 20 Myr or so for which we have good information regarding widespread occurrences of the spiriferid *Howellella* the shells have a maximum width of about 1 cm, until near the end of the Silurian, when a small increase in size begins to be evident. By the end of the Lower Devonian (another 15 Myr or so) some of the descendants of the Silurian *Howellella* such as *Acrospirifer*, *Euryspirifer* and *Fimbriospirifer* reach 8 cm in width (a rate of only a few mdarwins during 20 Myr followed by an average rate of about 250 mdarwins for 15 Myr!). The increase from a little more than 1 cm to 8 cm or so occurs with increasing rapidity during the Lower Devonian. *Atrypa* of the *A. "reticularis"* type have a maximum width during the Silurian of about 2 or 3 cm whereas in the Lower Devonian there is an increase to about 5 cm (no significant increase during the Silurian followed by an average rate of about 70 mdarwins during the Lower Devonian). This average rate of increase is far lower than that of the spiriferids, although it is significant. Taxa showing overall decrease in size are well known among such groups as the chonetids and stropheodontids during this same time interval, although in these groups far more show increases in size as predicted by Stanley's Fig. 3. Other genera such as *Skenidioides* and *Dicaelosisia* show no significant increase in size during the Silurian-Lower Devonian time interval.

Palaeontologists have found that during some time intervals related species of diverse groups tend to be larger than during other time intervals. In North America Middle Ordovician brachiopods tend to be significantly smaller than those of the uppermost Ordovician (Newell⁶ comments on this relationship). I have earlier⁷ mentioned the fact that

Fig. 1 Diagrammatic representation of upper and lower size limits for species belonging to a major taxon, with an indication of taxa increasing and decreasing their size phyletically from the overall, lower size characteristic of the major taxon.



Silurian brachiopods as a whole tend to be small relative to Lower Devonian brachiopods belonging to the same families and subfamilies (other groups such as bivalves show the same trend). Uppermost Devonian (Famennian)–Lower Carboniferous brachiopods are significantly smaller than those of the Upper Carboniferous–Permian. (Newell⁶ supports this conclusion with data from other groups as well.) Nicol's comments⁸ regarding the overall larger size reached by many Upper Cretaceous bivalves as contrasted with Lower Cretaceous and Jurassic ones is another good example. What explanation accounts for these non-random, time-correlated increases in size?

The more rapid the rate of phyletic evolution characteristic of a group the larger will be the number per unit time of species showing an increase in size, and the larger will be the number of species showing other evolutionary changes. Times of increasing overall rate of phyletic evolution for any major group of organisms will be the same time intervals when number, percentage and production rate of taxa increasing in size will be at a maximum. Conversely, the lower the overall rate of phyletic evolution characteristic of a group the smaller will be the number and percentage of taxa which achieve a larger size than is normal or modal for the group. It is noteworthy that an abrupt increase in phyletic rate commonly coincides initially with the brief upsurge in cladogenetic rate accompanying major provinciality increase¹.

A major terminal extinction event (such as those that occurred at the end of the Ordovician, the end of the Frasnian, the end of the Permian, and the end of the Cretaceous) will tend selectively to eliminate the larger, more specialised, more stenotopic species. There may then be a lengthy interval of relatively low overall rate of phyletic evolution, with few large species being produced, that is ultimately followed by an interval of higher overall rapid rate of phyletic evolution during which a higher number of large species is produced. Of course, there need be no long interval of low phyletic evolutionary rate and low number of large species. Silurian–Devonian brachiopods following the terminal Ordovician extinction event have a low overall rate of Silurian phyletic evolution with smaller number of large species, followed by a high rate of Lower Devonian phyletic evolution with large number and percentage of large species. The Permo–Carboniferous following the Late Devonian (terminal Frasnian) extinction event has a low rate of Famennian–Lower Carboniferous phyletic evolution with smaller number of large species followed by a high rate of Upper Carboniferous–Permian phyletic evolution with large number and percentage of large species. The Jurassic–Cretaceous data are similar, as detailed earlier.

For the neritic marine benthos considered here, which probably had planktotrophic larvae, there is no reason to think that numbers of larvae⁹ will differ markedly between individuals of large and small size belonging to various species. But a far smaller absolute number of larvae belonging to species of larger size will attain maturity than is the case for species of small size. As regards mammals it is obvious that their reproductive characteristics dictate much smaller broods and lower reproductive rates in species of large size than in those of small size. This correlation of reproductive rate with size in mammals should help to exaggerate the disparity in phyletic rate between large and small mammals in terms of population size. Clearly, the critical factor relating to rate of evolution and population size for mammals large and small, as well as invertebrates, is the number of individuals reaching the reproductive stage.

In principle the low population densities characteristic of abyssal benthos would suggest rapid phyletic evolution, as does the possibility that a non-planktotrophic larval habit is widespread, but the level of cosmopolitanism characteristic of many abyssal species may counteract this possibility, and

there is no suggestion that large species in most groups are more abundant in the abyss than on the shelf.

The relation between population size and taxon size (size of individuals in a taxon) also holds on land as indicated by Wolff's¹⁰ recent compilation of information for mammals. There is also a tendency for large mammals to evolve more rapidly than small mammals such as rodents. Maglio's¹¹ work on the elephants and Hecht's¹² consideration of the evolution of the polar bear are both highly consistent with an inverse relation between population size and rate of evolution.

The speed with which infraspecific changes in size may occur in conditions of small population size is underscored by the work of Foster¹³, Corbet¹⁴, Berry¹⁵, and Delany¹⁶, all of whom provide evidence indicating that small populations isolated on small islands from a large mainland population depart from normal size very rapidly. It takes little imagination, post-Darwin, to combine this information with other data showing the uniqueness of island faunas in many parts of the world and then to see the high correlation between rates of evolution and population size.

Hallam's conclusion that larger taxa have a shorter time duration geologically than smaller ones follows from the premise that species of large size are generally more stenotopic and therefore more susceptible to terminal extinction when faced with significant environmental perturbations. Hallam's conclusion accords well with the fossil record of the marine invertebrates known to me, and would certainly seem to agree well with what is claimed for terrestrial vertebrates. In other words, there is a survival premium based on eurytopy that accompanies moderate size for the species of any major taxon. It must be emphasised here that the shorter time duration of rapidly evolving large species commonly involves terminal extinction whereas the shorter time duration of small population endemic species, many of which may also be of small or moderate size, involves phyletic evolution, or phyletic extinction, if you will. Small population endemic species of large size will be subject to rapid phyletic evolution for the same reasons as endemic small species but the large species will be more prone to terminal extinction due to any environmental perturbations.

In conclusion, it seems to me that the inverse correlation of the rate of phyletic evolution and size of interbreeding population is consistent (in addition to items considered earlier¹) with the following.

(1) Evolutionary changes in animal size are chiefly non-linear. (2) Increasing rate of phyletic evolution correlates positively with increasing size. (3) For any group of animals, or between groups of animals, the number of individuals of any species decreases as the average size of individuals increases. (4) The number and percentage of large species in any major group is larger during times of rapid phyletic evolution than during times of slower phyletic evolution. (5) The above evidence indicates that during times of rapid phyletic evolution there tends to be an increased number and percentage of species evolving which are larger sized and have small populations; this in turn helps to further speed up rate of phyletic evolution.

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Synergistic effect of a pheromone and a kairomone on host selection and colonisation by *Ips avulsus*

WE report for the first time a synergistic effect on the pheromone response of a bark beetle species (Coleoptera: Scolytidae) by the pheromone of a sympatric species. *Ips avulsus* Eichh. and *I. grandicollis* Eichh. are two sympatric *Ips* species often found colonising the same host tree in the southern USA. Male *I. avulsus* produce the pheromone ipsdienol (2-methyl-6-methylene-2, 7-octadiene-4-ol)^{1,2} and both sexes are attracted to this compound in the field³. Male *I. grandicollis* produce the pheromone ipsenol (2-methyl-6-methylene-7-octene-4-ol)^{1,2,4} and both sexes respond to the S-(–)-isomer⁵. *I. avulsus* is known to respond in low numbers to both the crude attractant⁶ and infested host material⁷ of *I. grandicollis*. To investigate this relationship, a series of tests was carried out in east Texas. Two to four tree trunk-simulating olfactometers⁸ set 10 m apart adjacent to a loblolly pine stand at Lufkin, Texas, were baited with test material during the afternoon, when flight of *I. avulsus* occurred. Test materials included the optically pure enantiomers of ipsenol⁹, racemic mixtures of ipsenol and ipsdienol, and billets infested with male *I. avulsus* and *I. grandicollis*. All test materials used in a test series were offered simultaneously.

The results (Table 1) show that *I. avulsus* responded in significantly higher numbers to combinations of its own pheromone (ipsdienol) and that of *I. grandicollis* (ipsenol) than to either pheromone alone. This synergistic effect also occurred when natural attractants (infested billets) were

used in lieu of synthetic pheromones. Furthermore, the active optical fraction of ipsenol is the S-(–)-isomer, the same isomer which causes aggregation of *I. grandicollis*⁵.

Most scolytids, including *I. avulsus*, occupy temporary habitats; therefore, successful selection and colonisation of suitable host material is a critical phase in the life of these insects. The evolution of secondary attraction in order to fully exploit these habitats has been suggested¹⁰. Moreover, the synergistic effect of a pheromone–kairomone interaction, where the latter is produced by a related species, seems to be an additional adaptive strategy favouring successful host colonisation.

The mechanisms responsible for reproductive isolation in *I. avulsus* and *I. grandicollis* are unknown, but are probably related to differences in habitat preference within the host tree, specificity of mating behaviour, and genital incompatibility. With reference to *I. avulsus* and *I. grandicollis*, our findings³ do not support the hypothesis that specificity of pheromones among sympatric *Ips* species is an important mechanism in maintaining species integrity and preventing interspecific competition^{11,12}, nor the more recent suggestion that sympatric species of *Ips* further reduce interspecific competition through mutual pheromone inhibition¹³.

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Table 1 Field response of *I. avulsus* to olfactometers baited with synthetic and natural attractants

Test series	Test material	Release system*	No. of repetitions	Length of repetitions (min)	Average number of beetles trapped	Sex ratio (male–female)
1975	Ipsdienol	a	4	25	3.75	a† 1.5:1
I (2 September)	Ipsdienol + (+)–Ipsenol	a	4	25	5.00	a 1.9:1
	Ipsdienol + (–)–Ipsenol	b	4	25	34.75	b 2.9:1
	Ipsdienol + Ipsenol	a	4	25	22.50	b 2.8:1
	Ipsdienol	a	4	45	2.75	a 4.5:1
II (11 September)	(–)–Ipsenol	b	4	45	8.00	a 3.6:1
	Ipsdienol + (–)–Ipsenol	a	4	45	7.25	a 8.7:1
	Ipsdienol	b	4	45	7.25	a 8.7:1
III (11 September)	Ipsdienol + Ipsenol	a	5	40	9.40	a 3.3:1
	Ipsdienol	a	5	40	25.60	a 3.9:1
IV (18–19 September)	Ipsdienol	a	13	15	5.23	a 0.84:1
	Ipsdienol + Ipsenol	a	13	15	19.00	b 2.4:1
V (7–9 September)	<i>I. grandicollis</i> billet w 25 ♀					
	♀ + uninfested billet		5	60	2.00	a 9.0:1
	<i>I. avulsus</i> billet w 25 ♀					
	♀ + uninfested billet		5	60	8.20	a 2.2:1
	<i>I. avulsus</i> billet and <i>I. grandicollis</i> billet, each w 25 ♀		5	60	17.00	b 3.5:1
	Ipsdienol + Ipsenol	a	5	60	18.20	b 3.0:1

*a, Vial: 5 mm internal diameter × 30 mm; b, Capillary: 0.9 mm internal diameter × 10 mm.

†Means with different letters are significantly different at $P < 0.05$.

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Evidence that blackfly larvae can feed on particles of colloidal size

FILTRATION of colloidal particles has not previously been reported for animals living in flowing freshwaters. The observation that blackfly larvae (Diptera: Simuliidae) can filter such particles may alter our understanding of the nutrition of these suspension-feeding animals and also afford new possibilities for the control of the insect in areas where the adult females are vectors of onchocerciasis.

Blackfly larvae are equipped with specialised mouthparts, the cephalic fans, which, when extended into the current, trap suspended particles. At intervals the fans are folded and particles trapped on the fans are removed by the mandibles, passed to the cibarium and, thence, to the foregut. Recorded lengths of ingested particles vary between 0.5 and 300 μm with the majority in the range 20–100 μm for some Canadian larvae¹, and from 11.3 ± 1.8 to 15.1 ± 2.0 μm for several British species². That larvae can feed exclusively on fine particles has been demonstrated by rearing them to pupation on a diet of *Bacillus subtilis* (Ehr.) Cohn in distilled water³.

In the present investigation an aquarium tank (13 × 13 × 17 cm) was filled with distilled water and a circulating current created by an air pump. A concentrated suspension of polystyrene latex microspheres (Dow Chemicals) of diameter 0.091 ± 0.0058 μm was added to the water to give a suspension of less than 0.02% by volume. Large larvae (fifth instars and above, mainly of *Simulium nitidifrons* Edwards) were collected from a stream and placed in the aquarium for 24 h after which they were removed and killed in formalin.

The larvae were then cut into thick sections (~1 mm) using a scalpel and the surface of each viewed in a scanning electron microscope. In section, the gut contents were white and granular. When a little of this material was observed by transmission electron microscopy it was seen to consist of dense, three-dimensional aggregates often composed of many hundreds of latex microspheres.

Aggregations of microspheres were rarely observed in samples from the concentrated, or the dilute experimental suspension. Those aggregates which were observed consisted of few microspheres arranged in a single layer and probably resulted from drying the suspension on grids before examination by transmission electron microscopy. It is therefore concluded that the large three-dimensional aggregates found in the gut must form either on the cephalic fan rays, or as the result of 'packing' after the ingestion of single spheres.

It thus seems that blackfly larvae are able to feed on particles as small as 0.091 μm in diameter although the details of the feeding mechanism have yet to be fully understood. The use of particulate insecticides of 10 μm diameter against blackfly larvae has been demonstrated to be selective⁴. An insecticide preparation of smaller dimension would reduce sedimentation and allow the treatment to be even more selective.

The nutrition of blackfly larvae has not been studied in detail but fine particles, in addition to larger ones, may form an important part of the diet⁵. In 1909 it was suggested that dissolved organic matter was utilised in the nutrition of some aquatic animals⁶, a theory which has subsequently

been questioned. In the light of the present findings this theory may well have some basis in the case of blackfly larvae.

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Prochlorophyta as a proposed new division of algae

UNICELLULAR algae associated with ascidians from tropical Pacific shores have been reported by various biologists^{1–5}. They are bright green, generally spherical and about 10–20 μm in diameter, and they seem to have no clearly delimited nucleus or plastids. Such cells (identified as *Synechocystis didemni*), found associated with surfaces of *Didemnum* colonies on the Pacific coast of Mexico, have been shown by electron microscopy to be prokaryotic^{6,7}, which suggests that they are cyanophytes, that is, blue-green algae. Although all known blue-green algae (other than a few apochlorotic types) contain phycoerythrin, phycocyanin, or both, however, these ascidian symbionts are apple green and contain no detectable bilin pigments. Furthermore, like the eukaryotic algae in the divisions Chlorophyta and Euglenophyta, they contain two chlorophyll components, separable by chromatography and provisionally identifiable as chlorophylls *a* and *b* (ref. 8), whereas no cyanophytes are known to contain chlorophyll *b*. The assignment of *S. didemni* to any of the established algal divisions, therefore, presents a major taxonomic problem.

Algal cells found living inside the colonies of other didemnid ascidians at Enewetak Atoll, Marshall Islands, and on Coconut Island, Hawaii, have been recently studied by the same techniques and have proved to be very much like those of *S. didemni*. My colleagues and I (unpublished results) have confirmed in particular that those from *Diplosoma virens* (from Hawaii) are prokaryotic, contain chlorophylls *a* and *b*, contain no detectable bilin pigments (even after incubation for one or more days in nitrogenous media) and show no evidence of phycobilisomes on the thylakoids. In the light, in aerobic conditions, they can fix CO₂ and evolve oxygen. They must therefore be considered as algae (rather than as photosynthetic bacteria, which are normally anaerobic and, when illuminated, fix CO₂ without concomitant oxygen evolution). They cannot, however, be logically assigned to the Cyanophyta, the Chlorophyta, the Euglenophyta, or any other existing division of the algae.

In view of the fact that their peculiar combination of characteristics is common to algal symbionts from several different ascidians found in widely separated locations, it now seems appropriate to put such organisms in a new algal division, which I propose to call the Prochlorophyta. Since it will consequently be necessary to remove the type species, *S. didemni*, from the Cyanophyta, it can no longer be retained in the blue-green algal genus *Synechocystis*, in

spite of morphological similarities. The new division is formally described in Latin (requisite for all new plant taxa) as follows: *Prochlorophyta: algae procarioticae similes Cyanophytis, cuius differentes pro formatio chlorophyllarum binarum (a, b-que); pigmentia rutila aut caerulea absunt.*

(Formal descriptions of the new class, order, family, genus and type species will be presented elsewhere.)

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Chemotherapy of *Theileria parva* infection

EAST coast fever (ECF), the most important tick-borne disease of cattle in eastern and central Africa, is caused by the protozoan parasite *Theileria parva*. No successful therapy is available and mortality can reach 96% (ref. 1), although large doses of tetracyclines have some prophylactic effect². The search for an active drug has been hindered by the lack of a laboratory model of the infection. An *in vitro* screening process has been established, using the infected bovine lymphoid cell culture methods developed by Malmquist *et al.*³. This has now shown that 2-hydroxy-3-(8-cyclohexyloctyl)-1,4-naphthoquinone (Menotone, Sterling-Winthrop) has a high level of anti-theileria activity *in vitro*. Subsequent tests in cattle artificially infected with ECF confirmed the activity of this compound against the disease.

Screening was conducted with the E174 line of *T. parva*-infected bovine lymphoid cells grown in glass, screw-cap tubes, in the supplemented Eagle's minimum essential medium described by Malmquist *et al.*³. The tubes were 'gassed' with a mixture of 5% carbon dioxide in air. Compounds under study were dissolved or suspended in a minimum volume of ethanol, then appropriately diluted with sterile culture medium before addition to the cultured, infected cells. Each test was duplicated. At the beginning of a test the cultures contained 3×10^5 cells ml⁻¹. Tubes were incubated for 48 h at 37 °C, with gentle agitation. The growth of untreated cultures was determined using a haemocytometer, and the growth of drug-treated cultures was estimated with reference to this value, using a five-point scale. Control cultures usually contained 8×10^5 – 10×10^5 cells ml⁻¹ after 48 h, and this was scored 5⁺. A tube in which there had been no increase in cell numbers was scored 3⁺, and one in which all cells were dead was scored 1⁺. This gave an indication of the toxicity to the host cells of the compounds being tested. To assay the effect on the parasite the tubes were centrifuged at 500g for 5 min, and a smear was made from the pellet on a glass microscope slide. This was stained by the method of Field and Sandosham⁴ and the percentage of infected cells was counted.

A wide range of anti-parasitic compounds was tested *in vitro*, including several which had been reported (without confirmation) to show activity against ECF *in vivo*. The results are summarised in Table 1.

Only Menotone showed appreciable activity, and was selected for further testing in cattle infected with ECF. Fourteen high grade Jersey heifers, weighing about 90 kg, were divided randomly into two groups of seven. They were infected with *T. parva* (Muguga) by injection of 1 ml of a stabilate prepared from ground up, infected ticks⁵, *Rhipi-*

cephalus appendiculatus, in front of the right ear. The course of subsequent infection was monitored by reading rectal temperatures daily, and by examination of Giemsa-stained smears of biopsy material, from the local drainage lymph node and the contralateral prescapular lymph node, for the presence of macroschizonts and microschizonts. Stained blood smears were examined for the presence of piroplasms in the erythrocytes.

Menotone was formulated as a 10% solution in a mixture of 20% dimethylsulphoxide and 80% corn oil. The compound was injected intravenously into one group of seven cattle, while the remaining seven were kept as untreated controls. The first dose (5 mg kg⁻¹) of Menotone was injected on the first day on which a temperature of 39.5 °C was recorded and macroschizonts were detected in the local drainage lymph node. A further five daily doses of 1 mg kg⁻¹ were given, making a total dose of 10 mg kg⁻¹.

In the untreated cattle the typical ECF syndrome developed, and six died. The seventh became severely ill but recovered. In all seven treated with Menotone the progress of the disease was soon halted; temperatures returned to normal levels and schizonts could not be detected in lymph-node smears. Within 48 h of the first injection of Menotone, schizonts seemed to be degenerating and were apparently destroyed within the lymphoid cells. An animal was regarded as 'recovered' 3 d after schizonts were last observed in lymph-node smears. The results are summarised in Table 2.

On day 38 after their original infection, all eight surviving cattle were challenged with a similar injection of stabilate. All were highly resistant, although small numbers of schizonts were detected in the one surviving control and in two of the animals treated with Menotone. Six normal cattle which received the challenge dose reacted severely and died.

Menotone therefore seems to have a high level of activity against *T. parva* in artificially infected cattle, when injected after the infection has become established. In an earlier experiment Menotone was injected on the day of infection at 5 or 2.5 mg kg⁻¹ and on the subsequent 9 d at 1.0 or 0.5 mg kg⁻¹, a total of 14 or 7 mg kg⁻¹. In groups of five cattle infected with *T. parva* stabilate the disease was almost totally suppressed by these treatments, and no cattle

Table 1 Activity of compounds against *Theileria parva* *in vitro*

	Concentration (mg ml ⁻¹)	Cell growth	% Cells infected
Menotone	1.0	4 ⁺	22
	0.1	4 ⁺	24
	0.01	5 ⁺	44
	0.001	5 ⁺	72
	0.0001	5 ⁺	83
	1.0	5 ⁺	97
Chloroquine	1.0	5 ⁺	90
Mepacrine	1.0	5 ⁺	96
Pamaquine	10.0	4 ⁺	98
Primaquine	10.0	2 ⁺	98
Cycloguanil	1.0	4 ⁺	97
Pyrimethamine	10.0	2 ⁺	100
Amprolium	10.0	5 ⁺	100
Decoquinat	10.0	5 ⁺	100
Diaveridine	10.0	2 ⁺	100
Clopidol	10.0	5 ⁺	98
Metronidazole	10.0	5 ⁺	97
Robenidene	10.0	5 ⁺	88
Sulphaquinoxaline	10.0	5 ⁺	88
Amicarbalide	10.0	5 ⁺	81
Imidocarb	100.0	3 ⁺	97
Phenamidine	10.0	5 ⁺	85
Diminazine diacetate	100.0	3 ⁺	100
Suramin	10.0	5 ⁺	88
Oxytetracycline	100.0	4 ⁺	97
Ethanol	(0.01 %)	5 ⁺	97
None	—	5 ⁺	98

Table 2 Activity of Menoctone against *T. parva* in cattle

	Macroschizonts	Temperature 39.5 °C	Recovery	Days to: Death
Treated (0/7 died)	9.0±0.0	7.7±1.7	18.4±5.0	—
Untreated (6/7 died)	8.8±0.4	8.8±1.3	27	18.5±2.4

The total dose of Menoctone was 10 mg kg⁻¹. Figures show group means and standard errors.

became clinically ill. Three of five controls died, and a fourth was severely affected. The fifth developed only mild ECF. On challenge, the cattle dosed with Menoctone at 14 mg kg⁻¹ were highly susceptible, while those dosed with 7 mg kg⁻¹ were partially resistant, suggesting that the drug acted very early in the course of infection, before immunity had become established.

The mode of action of Menoctone on *T. parva* is not clear, although it seems to act against the schizont and possibly earlier stages of the cycle. Peters⁶ suggested that the antimalarial activity of Menoctone may be due to a blocking of the biosynthesis of coenzyme. The apparent destruction of the *T. parva* schizont within the host lymphoid cells, both *in vitro* and *in vivo*, agrees with this suggestion. The number of different parasites against which Menoctone is active, which includes coccidia⁷, and *Theileria annulata in vitro* (N.M., unpublished results) indicates action on a fundamental and widely occurring biochemical pathway. Of the 20 anti-parasitic compounds tested against *T. parva in vitro*, only Menoctone was active at levels not toxic to the host cells. This suggests that the metabolism of *T. parva* is closely adapted to that of the host cell. The difficulty of finding a compound which will exploit differences between the metabolism of the parasite and its host cell perhaps indicates why no effective therapeutic compound is available for this economically very important disease.

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to incorporate AD^{3,6-8}, prompting suggestions that the AD-resistant phenotype is determined by changes in plasma membrane function that reduce cellular permeability to this drug. Support for this proposed mechanism of drug resistance has been provided by experiments showing that treatment of AD-resistant cells with membrane-active detergents, to increase plasma membrane permeability, enhances AD uptake by resistant cells and reduces the concentration of AD required to inhibit cell growth^{8,9}. This approach, while instructive, has the disadvantage that the detergent causes significant alteration in cell viability and also impairs cell growth⁸. We now report a new method for enhancing drug uptake by permeability restriction, drug-resistant cells which uses lipid vesicles as carrier agents to introduce AD directly into cells. As reported elsewhere^{10,11}, negatively-charged lipid vesicles composed of lipids or lipid mixtures that are “fluid” at 37 °C are incorporated into cultured cells by fusion with the cellular plasma membrane. Entrapment of drugs inside such vesicles and subsequent fusion of the vesicles with cells offers a potential method for bypassing the plasma membrane transport barrier to introduce drugs directly into the intracellular compartment. Since vesicles are not cytotoxic¹⁰⁻¹², their use as drug carriers allows direct evaluation of the cellular response to the introduced drug, free from the complications^{8,9,13} associated with the use of detergents. The results presented here indicate that uptake of vesicles containing AD by AD-resistant cells produces inhibition of cellular RNA synthesis and cell growth at AD concentrations that have no effect on cells when added as free drug to the culture medium.

The AD-resistant Chinese hamster cell line, DC3F-ADX, (provided by Dr J. Biedler, Sloan-Kettering Memorial Institute) was grown in Eagle's MEM supplemented with 10% foetal calf serum. The isolation, characterisation and properties of this cell line have been described elsewhere^{4,7}. Cells were grown routinely in MEM containing AD at 10 µg ml⁻¹ to prevent emergence of AD-sensitive variants, but grown in medium without AD for 10–15 d before use in experiments. Vesicles were prepared from a lipid mixture containing 1 µmol of phosphatidylserine, 9 µmol of phosphatidylcholine and 10 µmol of cholesterol which were mixed in chloroform, evaporated to dryness in a vacuum and suspended in 1.0 ml of calcium- and magnesium-free phosphate-buffered saline (PBS) containing different concentrations of AD. This mixture was used since vesicles of this composition are incorporated into cells by fusion with the plasma membrane^{10,11}. All lipids were purified and characterised in our laboratory and were chromatographically pure¹⁴. Trace amounts of ³H-AD (Amersham Searle, 7 Ci mmol⁻¹) were added to the lipid mixture before evaporation as a marker for quantitating drug capture inside vesicles. The suspension of lipids and AD in PBS was mixed vigorously on a Vortex shaker for 10 min at 37 °C after which the preparation was sonicated in a bath type sonicator for 1 h at 37 °C under nitrogen to produce unilamellar vesicles¹⁴. The sonicated preparation was equilibrated for 30 min at room temperature and then passed through a Sephadex G-50 column to separate vesicles containing AD from untrapped AD. Capture of AD by vesicles was then determined by the extent of recovery of ³H-AD in association with vesicles as a fraction of the ³H-AD present in the original lipid suspension in buffer. When

Drug-containing lipid vesicles render drug-resistant tumour cells sensitive to actinomycin D

DEVELOPMENT of cellular resistance to actinomycin D (AD) has been reported in tumour cell populations *in vivo*^{1,2} and *in vitro*³⁻⁶. Such cells have a lower capacity than normal

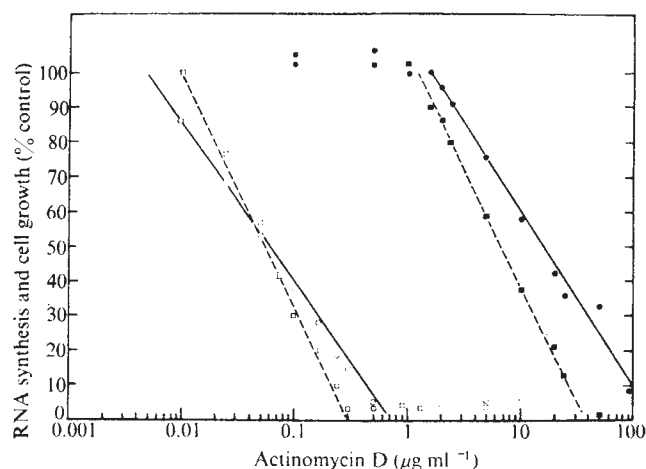


Fig. 1 Effect of free AD and AD entrapped within lipid vesicles on cellular RNA synthesis (—) and growth (----) of AD-resistant DC3F-ADX hamster cells. Closed symbols represent cells treated with free AD and open symbols represent cells treated with lipid vesicles containing different concentrations of AD. Exponentially growing cell populations cultured in 60-mm plastic Petri dishes (cell density $20 \times 10^4 \text{ cm}^{-2}$) were incubated for 2 h at 37°C in MEM containing either free AD at the indicated concentrations or lipid vesicles (200 nmol of lipid per 10^6 cells) containing different concentrations of AD. Vesicles were prepared as described in the text. The cultures were then washed with PBS to remove the drug or drug-containing vesicles and the ability of the cells to synthesise RNA and to proliferate was determined. Control cell cultures were incubated with drug-free medium for 2 h at 37°C (see text for additional control experiments). To measure cellular RNA synthesis the drug-treated cultures were incubated in fresh medium containing ^3H -uridine ($1.5 \mu\text{Ci ml}^{-1}$) and the amount of cell-associated radioactivity incorporated into a trichloroacetic acid-insoluble fraction was measured after 17 h and expressed as a percentage of the incorporation occurring in replicate control cultures not exposed to AD. The ability of drug-treated cells to proliferate during 24 h was determined by the growth assay described previously⁹ and the results were expressed as a percentage of the growth occurring in untreated control cultures. Each point represents a mean value from measurements of four cultures at the indicated drug concentration.

the starting AD concentration in buffer was $600 \mu\text{g ml}^{-1}$, approximately 10% was captured by the vesicles. The efficiency of drug capture increased at lower starting concentrations of AD, so that at a starting dose of $17 \mu\text{g ml}^{-1}$ or lower, approximately 60% of available drug was trapped by the vesicles. This indicates considerable binding of the drug to the vesicles in addition to passive capture within the aqueous interior of the vesicle, which can be calculated as approximately 2% of the total. After separation of the vesicles on Sephadex G-50, the lipid concentration was $2 \mu\text{mol}$ of phosphate per ml with an incorporated AD concentration of $12 \mu\text{g ml}^{-1}$ ($5 \times 10^{-5} \text{ M}$) or less as indicated below. For comparison of cellular sensitivity to free AD and AD entrapped within vesicles, free AD was obtained from the effluent of the same column of Sephadex G-50 as the vesicles and diluted to the same concentration as vesicle-entrapped AD by dilution in PBS to give identical ^3H -AD counts. The free drug elutes as a delayed peak completely separate from the lipid peak.

Previous work has shown that the present DC3F-ADX cell line is highly resistant to AD, requiring 250 times more AD to produce effective inhibition ($>75\%$) of cellular RNA synthesis than the wild-type sensitive cell line (DC3F) from which it was derived^{4,7}. Even when cultured in MEM containing AD at $50 \mu\text{g ml}^{-1}$, limited RNA synthesis is still detectable in resistant cell populations (Fig. 1). But treatment of replicate populations of resistant cells with lipid vesicles containing different concentrations of AD resulted

in effective inhibition ($>75\%$) of cellular RNA synthesis at a drug concentration of $0.25 \mu\text{g ml}^{-1}$ (Fig. 1), a 200-fold decrease over the concentration of free drug required to produce the same effect. Measurement of the incorporation of ^3H -AD into resistant cells revealed that cells treated with vesicles containing ^3H -AD incorporated five to six times more AD than replicate cultures exposed to identical concentrations of free ^3H -AD (results not shown).

Enhancement of AD uptake into drug-resistant cells by vesicles also produced a significant reduction in the concentration of AD required to inhibit cell proliferation (Fig. 1). The concentration of drug required to reduce cell growth to 50% of that occurring in untreated control cultures was reduced from $7.2 \mu\text{g ml}^{-1}$ for cells exposed to free AD to $0.062 \mu\text{g ml}^{-1}$ for cells treated with vesicles containing AD (Fig. 1).

Control experiments in which resistant DC3F-ADX cells were incubated with similar numbers of vesicles without AD established that vesicle uptake *per se* had no effect on cellular RNA synthesis or cell growth (not shown). Further experiments in which resistant cells were incubated with vesicles without entrapped AD, but in medium containing free AD, also failed to alter cellular RNA synthesis or cell growth from that seen with free AD alone (results not shown). This suggests that vesicle uptake by cells does not alter significantly the transfer of free AD across the cellular plasma membrane.

The results in Fig. 1 thus indicate that vesicle-mediated enhancement of drug uptake by resistant DC3F-ADX cells substantially increases cellular sensitivity to AD. These findings support previous proposals^{3,5,7-9} that the AD-resistant phenotype results from a decreased cellular capacity to incorporate AD, produced by a reduction in the permeability of the cellular plasma membrane to this drug. But the fact that vesicle-mediated delivery of AD increases the uptake of AD five- to sixfold over that achieved with free drug, and yet is accompanied by a 200-fold reduction in the drug concentration needed to inhibit cell growth and RNA synthesis, raises the question of whether the vesicles deliver AD to a specially sensitive site of drug action within the cell. For example, AD binds to lipid-rich cellular components and the delivery of AD by the vesicle route might bypass some of this nonspecific binding.

The potential value of lipid vesicles as carriers for introducing pharmacological agents into cells has been proposed before¹⁴⁻¹⁹. Although our experiments were restricted to AD-resistant tumour cells, there is no *a priori* reason why lipid vesicles could not be used similarly to enhance the uptake of other cytostatic and cytotoxic drugs by resistant tumour cells. Resistance of mammalian tumour cells to methotrexate, daunomycin, adriamycin, *Vinca* alkaloids and the terephthalanides and related agents is believed to have a basis similar to AD resistance in that resistant cells fail to incorporate sufficient of these drugs due to reduced drug transfer across the plasma membrane²⁰.

Recent studies in this laboratory have also shown that vesicles can be used as drug carriers to increase the cytotoxic potency of nucleoside and nucleotide analogues. Ara-CTP, which is not ordinarily taken up by cells, has been introduced into tumour cells using vesicles as drug carriers with a significant increase in cytotoxic activity compared with ara-CTP alone (unpublished results of E. Mayhew, D. P., Y. M. Rustum, C. Dave).

Vesicle-mediated delivery of drugs into drug-resistant tumour cells might therefore provide a possible novel approach for cancer chemotherapy *in vivo*, particularly if "recognition ligands" could be included in the vesicle membrane to induce preferential uptake of vesicles by tumour cells. Some initial progress in this direction has already been achieved *in vitro*, where incorporation of cell-specific immunoglobulins into vesicle membranes resulted

in a significant increase in vesicle uptake by the specific cell types to which the vesicles were "targeted"²¹.

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Selective cytotoxicity for transformed 3T3 cells

THE therapeutic usefulness of many anti-cancer agents depends on their selective toxicity for cells in the S (DNA synthesising) phase of the cell cycle¹. Thus, rapidly proliferating cancer cells are more effectively killed by these agents than are normal cell populations, which have a lower proportion of cells in S. In theory, it should be possible to reduce the toxicity of such agents significantly by further slowing the entry of normal cells into S. We have tested this possibility *in vitro* by exploiting the ability of cyclic AMP derivatives to inhibit the entry into S phase of 3T3 cells, but not their malignant counterparts, transformed by SV40.

Analogues of cyclic AMP have been reported to slow the growth of both 3T3 and SV403T3 cells. Such drugs reduce the saturation density of untransformed cells, arresting them in the G₁ phase of the cell cycle²⁻⁴. As suggested by others⁵⁻⁶, however, and confirmed in our experiments, these drugs do not reduce the proportion of SV403T3 cells synthesising DNA. Thus we predicted that normal 3T3 cells, accumulated in G₁ after exposure to cyclic AMP derivatives, would be protected from the cytotoxicity of anti-cancer drugs that act during the S phase, while transformed cells would not. Experiments presented here substantiate this prediction.

Initially, we verified that the addition of dibutyl (db) cyclic AMP plus 1-ethyl-4-hydrazine-1-H-pyrazolo-(5,4-6)-pyridine-5 carboxylic acid ethylester (SQ20006), a potent inhibitor of cyclic nucleotide phosphodiesterase⁷, reduces the proportion of 3T3 cells in S from 70% to 13% but not in SV403T3 cells (Table 1).

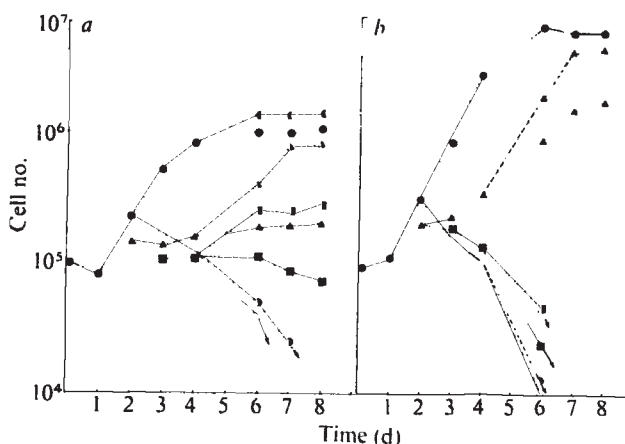
We investigated whether the addition of compounds which increase cyclic AMP protects 3T3 but not SV403T3 cells from hydroxyurea, a cytotoxic drug that acts during S. Both cell types were plated on plastic Petri dishes (5 cm dia-

meter) at a density of 10⁵ per dish. After 24 h some cultures received 0.2 mM db cyclic AMP and 0.2 mM SQ20006 and incubation continued for 24 h to allow the 3T3 (but not SV403T3) cells to pass out of the S phase. Then 2 mM hydroxyurea was added to cultures pretreated and not pretreated with cyclic AMP derivatives. Figure 1 illustrates the growth curves obtained in a typical experiment. Cultures of 3T3 cells reached saturation density at about 10⁶ cells per dish, as expected for this cell line (Fig. 1a). Hydroxyurea efficiently killed the control cells, while a substantial proportion of the cells pretreated with cyclic AMP analogues remained attached to the dish and appeared to survive the toxic treatment. When media containing the drugs were replaced by fresh media, the cultures treated with hydroxyurea alone were damaged irreversibly, as judged by their inability to recommence growth. Conversely, growth resumed in the cultures pretreated with analogues of cyclic AMP. Furthermore, when the latter were replated at a lower cell density, they grew at a rate and reached a final saturation density similar to the original 3T3 cells (not shown).

The growth curves obtained with SV403T3 cells are shown in Fig. 1b. Control cells grew rapidly and reached very high densities before sloughing off from the dish. Addition of db cyclic AMP plus SQ20006 reduced the growth rate markedly, but, in contrast to the results in the 3T3 cells, hydroxyurea killed SV403T3 cells whether or not the cultures were previously exposed to agents that increase cyclic AMP (Fig. 1b). The experiments depicted in Fig. 1 have been repeated several times with similar results.

Since assessments of cell survival based on measurements of cell number can be complicated by the persistence of dead cells attached to the dish, we measured the ability of the cells to form colonies after treatment with combinations of drugs and for times similar to Fig. 1. Cells were exposed to the drugs for different times, washed and plated at different dilutions into medium without drugs. Figure 2

Fig. 1 Effect of cyclic AMP-increasing agents, hydroxyurea or both on the growth of 3T3 (a) and SV403T3 (b) cells. Both cell lines were seeded at 10⁵ cells per 5 cm Nunc Petri dish containing 5 ml of Dulbecco's modified Eagle's medium supplemented with foetal calf 10% serum, penicillin (100 U ml⁻¹) and streptomycin (100 µg ml⁻¹). After 24 h, some cultures (▲ and ■) received 0.2 mM db cyclic AMP and 0.2 mM SQ20006. After a further 24 h, both untreated and treated cultures received 2 mM hydroxyurea (■ and ○). The control cultures (●) were treated identically but without drugs. After 48 h, the medium of some cultures of each series was replaced by regular fresh medium (broken lines). Cells were counted by removing the cells from the dish with a trypsin solution (0.05% trypsin in Ca²⁺-Mg²⁺-free phosphate-buffered salt solution with EDTA) and counting a portion of the resulting cell suspension in a haemocytometer. Each point represents an average of two determinations.



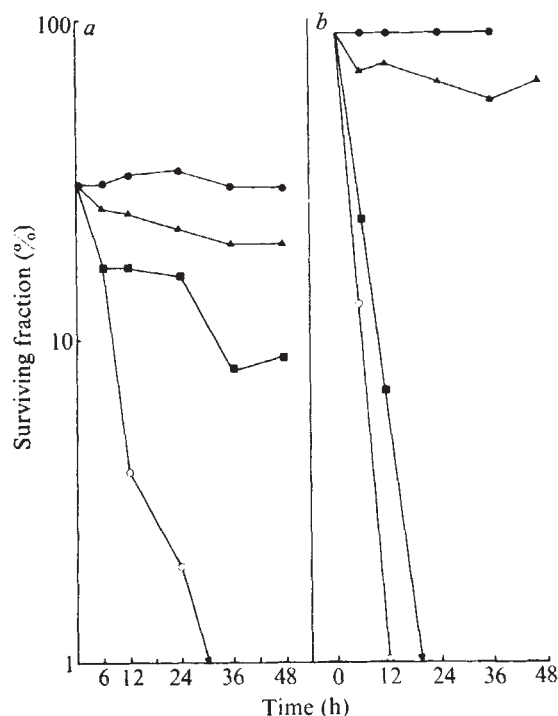


Fig. 2 Colony-forming ability of 3T3 (a) cells and SV403T3 (b) cells exposed to drug combinations. Both cell lines were plated (2×10^5) on 90-mm Nunc Petri dishes to ensure rapid growth. The drugs were added as described in the legend of Fig. 1, that is, agents that increase cyclic AMP 24 h after plating (▲ and ■) and hydroxyurea after a further 24 h (● and ○). Control cultures (●) received medium without drugs. After addition of hydroxyurea, the cultures were trypsinised at the times indicated in the abscissa, counted and plated at different cell densities in regular control medium without drugs. The cultures were incubated for 7–10 d, fixed, stained and the colonies formed counted. The data are expressed as percentage of initial cell inoculum.

shows that hydroxyurea killed both 3T3 and SV403T3 cells. But there was a considerable difference in the presence of cyclic AMP analogues; the 3T3 cells became much less susceptible to hydroxyurea toxicity than SV403T3 cells. These experiments were consistent with the growth curves shown in Fig. 1 and further substantiate the selective protection of 3T3 cells by cyclic AMP.

A set of experiments verified the specificity and dose-response of the compounds used. A gradual increase in protection was obtained when db cyclic AMP was changed from 0.05 to 0.4 mM. In addition, 0.5 mM isobutylmethylxanthine and 0.2 mM 8Br cyclic AMP could be used to replace SQ20006 and db cyclic AMP, respectively, as the protecting agent. Furthermore, similar results were obtained when hydroxyurea was replaced by cytosine arabinoside (10, 25 and 50 $\mu\text{g ml}^{-1}$) although the reversibility was less evident in these experiments.

Our results show that hydroxyurea kills SV403T3 cells

Table 1 Effect of db cyclic AMP plus SQ20006 on DNA synthesis of 3T3 and SV403T3 cells

Additions	3T3	SV403T3
Nothing	75	99
Db cyclic AMP (0.2 mM)	70	99
SQ20006 (0.3 mM)	40	96
Db cyclic AMP plus SQ20006	13	97

The cells (5×10^4) were plated in Nunc dishes (35 mm diameter) in Dulbecco's modified Eagle's medium. The cultures were exposed to the compounds for 24 h and then ^3H -thymidine ($5 \mu\text{Ci ml}^{-1}$) was added for a further 24 h. At the end of incubation, the cultures were prepared for autoradiography. The data represent labelling indexes (% labelled nuclei) recorded by two independent operators.

but not 3T3 cells when the cultures are pretreated with compounds that increase cyclic AMP. This phenomenon might reflect a rather general inability of fibroblasts transformed by polyoma or SV40 viruses to accumulate in a viable G_1 state in many non-optimal conditions⁸⁻¹². Alternatively, transformation by such viruses might alter the mechanisms of action of cyclic AMP, that is protein kinases¹³. Whatever the explanation, our findings have two interesting implications. First, they suggest an experimental system to select 'revertants' of SV403T3 cells which regain the ability of being blocked in G_1 by cyclic AMP and protected from S cytotoxic agents. Such variants would make possible further investigation of the genetic control of cyclic AMP action and the mechanism(s) of growth regulation in G_1 . Second, the data suggest a new experimental approach for a rational chemotherapy, namely, the use of compounds that protect normal cells. It has been found that compounds like caffeine, streptovitamin A¹⁴ and cytochalasin B¹⁵ protect normal, but not malignant, human and hamster cells from phase-specific cytotoxic drugs. Taken together, these findings indicate that differential protection of normal cells is feasible and it should be given serious consideration for accomplishing selective killing of at least some types of malignant cells.

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Aerobic glycolysis during lymphocyte proliferation

GLYCOLYSIS, for most mammalian cells, is only a prelude to the complete respiratory oxidation of glucose. Lactate production is usually barely, if at all, detectable in aerobic conditions¹. Consequently, when Warburg^{2,3} observed that various tumours showed active aerobic glycolysis, he postulated that defective tumour cell respiration was the reason and was, moreover, the basic difference between normal and cancer cells. Although aerobic glycolysis by tumour tissue has been confirmed many times, defective respiration in cancer cells has not been established⁴. The failure to uncover a respiratory defect in cancer cells has led to other explanations. It has been suggested, for example, that aerobic glycolysis is linked to cell growth rather than to malignancy⁵, and for several hepatomas a correlation could be made between the amount of lactate produced and the cell doubling time^{6,7}. It follows that if aerobic glycolysis is related to cell growth, it might be possible as much in normal as malignant cells. There is some evidence for this. Aerobic glycolysis has been noted in proliferating fibroblasts during the period of maximum increase in cell numbers^{8,9}. Human lymphocytes have shown an increase in respiration and also produced lactate when stimulated by the mitogen phytohaemagglutinin^{10,11}. In chick embryo and skeletal muscle fibroblasts^{12,13}, glycolysis increased during log phase growth, but several factors seemed to influence lactate

production. Medium composition, aggregation state of cells, culture pH and cell density were all considered more important in determining glycolytic activity than growth rate. It should be noted, however, that these factors are also important to the proliferative rate of these cells. We believe that the important question is not whether culture conditions can influence lactate production, but rather whether glycolysis is linked to cell division.

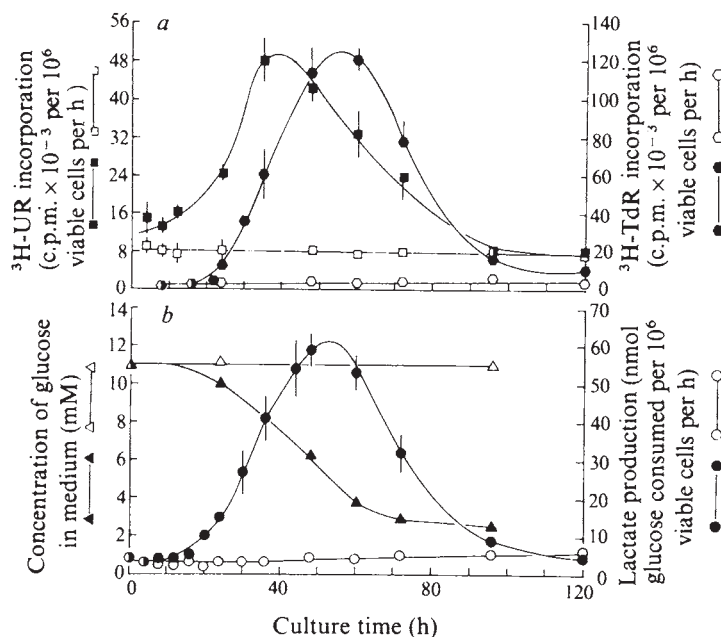


Fig. 1 The temporal relationship of macromolecular synthesis to aerobic glycolysis and glucose utilisation in mouse lymphocytes. Cells collected from mouse (C57BL/6 $\times$ DBA/2F₁ hybrids, 8–12 weeks old from Jackson Laboratory) spleens by gentle homogenisation were treated with 0.87% NH_4Cl to lyse erythrocytes. The resulting cell suspensions were 94–97% small lymphocytes by morphology. The cells were washed and resuspended in RPMI 1640 medium with 2% pooled human serum, 2 mM L-glutamine, penicillin (100 U ml^{-1}) streptomycin (100 mg ml^{-1}). The cell density was adjusted to 5×10^6 cells per ml and 25 ml samples were placed in plastic Petri dishes ($100 \times 15 \text{ mm}$) and incubated at 37°C under 5% CO_2 in a humidified National incubator. Mitogenic stimulation was produced by con A ($2 \mu\text{g per ml}$ of culture). Viability was determined by Trypan blue exclusion. The closed symbols represent con A-stimulated cells and the open symbols control cells. The vertical bars at the individual time points represent 1 s.e.m. For those points without the bars, the s.e.m. is smaller than the vertical dimension of the symbols. *a*, DNA synthesis ($\bullet$, $\circ$) was assayed by the incorporation of $0.5 \mu\text{Ci}$ (6 Ci mmol^{-1}) ^3H -thymidine (^3H -TdR); RNA synthesis ($\blacksquare$, $\square$) by the incorporation of $0.5 \mu\text{Ci}$ (6 Ci mmol^{-1}) ^3H -uridine (^3H -UR), and protein synthesis (not depicted because the curve is superimposable on the RNA curve) by $1 \mu\text{Ci}$ (59 Ci mmol^{-1}) ^3H -leucine into trichloroacetic acid (TCA)-precipitable material. For these labelling studies, at selected time points, 0.2-ml samples of the cell suspensions were transferred to microculture plates and labelled for 1 h. The assays were terminated using a microculture harvester; the cells were precipitated with 5% TCA, deposited on glass fibre filters presoaked with the appropriate unlabelled precursors and washed. After drying, the filters were immersed in scintillation fluid and counted in a Beckman L-230 liquid scintillation counter at a 30% counting efficiency. Each point is compiled from the means of triplicate determinations from four representative experiments. *b*, Lactate production ($\bullet$, $\circ$) was assayed at selected culture time points on cells washed and resuspended in glucose-free RPMI 1640 medium. Duplicate 0.5-ml samples of washed cells were incubated for 30 min at 37°C in $5 \text{ mM } ^{14}\text{C}$ -glucose ($80 \mu\text{Ci mmol}^{-1}$), centrifuged and 0.4 ml of the supernatant was added to $0.5 \text{ ml } 20\% \text{ CuSO}_4 \cdot 5\text{H}_2\text{O}$ followed by 2 ml of H_2O and sufficient $\text{Ca}(\text{OH})_2$ to form a bright, blue, fluffy precipitate. After 30 min at 25°C , the mixture was centrifuged and 1.8 ml of the supernatant was taken for a second $\text{CuSO}_4/\text{Ca}(\text{OH})_2$ treatment. Supernatant (1 ml) from the second treatment was mixed with 5 ml of scintillation fluid containing Triton X-100 and counted at 85% efficiency. Glucose concentrations ($\blacktriangle$, $\triangle$) were determined colorimetrically. Duplicate 1-ml samples of culture medium or glucose standards were added to 1 ml of 5% phenol and 5 ml of concentrated H_2SO_4 . The absorbance at 490 nm was read in a Beckman spectrophotometer 30 min later.

So far no systematic study of the relationship of the appearance of aerobic glycolysis to cell proliferation or the phases of the cell cycle has been reported. This report presents evidence in normal lymphocytes that aerobic glycolysis not only is associated with cellular proliferation, but more specifically is temporally related to DNA synthesis.

Lymphocytes enter a proliferative cycle after stimulation by concanavalin A (con A). We have found, as have others previously, that among the early changes, the rates of protein and RNA synthesis were increased within 2–4 h (Fig. 1a)¹⁴. The increase in RNA and protein synthesis continued and both reached a maximum about 40 h after con A addition and decreased thereafter. DNA synthesis, on the other hand, did not appear until about 20 h and peaked between 50 and 60 h after stimulation (Fig. 1a). After this maximum, DNA synthesis fell and returned to baseline level. It is interesting that the decline in DNA synthesis, as well as RNA and protein synthesis, could not be prevented by frequent changes of medium, serum replenishment or the continued presence of con A. Thus, these cells undergo a discrete proliferative response (one to two cell divisions) to stimulation by con A and, unlike established lines or tumour cells, retain control of their division.

Glycolytic activity in lymphocytes during the proliferative response was assessed by measuring the production of ^{14}C -lactate from ^{14}C -glucose at selected times after stimulation by con A (ref. 15). Previous experiments had established that the rate of ^{14}C -lactate production was linear with pulse times up to 1 h and reached a plateau at a glucose concentration of 2 mM. When compared with a direct determination of lactate accumulated, this assay has the advantages of short measurement intervals, the elimination of background accumulation in the medium and the reduction of lactate reutilisation. This assay does, however, depend on the utilisation of exogenous glucose. Other sources, for example glycogen, might provide glucose for glycolysis. To investigate this possibility, the lactate released into the medium was measured directly by the lactate dehydrogenase assay¹⁶. The problems inherent in this method include a longer sampling time and presumed increased reutilisation. Nevertheless, the results of these determinations closely approximated those obtained with the ^{14}C -lactate assay (Fig. 1b) and only the latter will be discussed.

Lactate production changed considerably during lymphocyte proliferation and from the data presented (Fig. 1a and b) it can be seen that for the first 20 h of stimulation by con A, there was a small but significant increase in lactate production. With the onset of DNA synthesis at approximately 20 h, however, it increased sharply. Aerobic glycolysis reached a maximum value about 20 times greater than that of control cells by 50–60 h, then fell as DNA synthesis declined. Although these cells were not synchronised, it has been shown frequently that the amount of ^3H -thymidine incorporation correlates with the percentage of cells synthesising DNA¹⁷. The lactate production curve, in turn, coincides temporally with that of ^3H -thymidine incorporation, suggesting that it is the cells in S phase that generate most of the lactate. Furthermore, it should be noted that in these studies the cells progress through G_0 , G_1 and S without being subjected to the artefacts caused by synchronisation techniques. Whether or not the intense aerobic glycolysis which accompanies DNA synthesis continues into G_2 and M, however, cannot be determined without cell synchronisation.

The increase in glycolytic activity would be expected to be accompanied by a greater glucose consumption. Figure 1b shows that lymphocytes stimulated by con A before DNA synthesis utilised glucose slowly, but with the onset of DNA synthesis the rate sharply increased. Glucose consumption subsequently tapered off as ^3H -thymidine incorporation diminished, although concentration of glucose in the medium was still above saturation level (2 mM) for lactate production. There was no substrate limitation for glycolysis in these culture conditions, and in other experiments replenishment of the glucose to the original concentration (11 mM) neither increased glucose utilisation measurably nor prolonged DNA synthesis.

The close correlation of DNA synthesis and aerobic glycolysis is

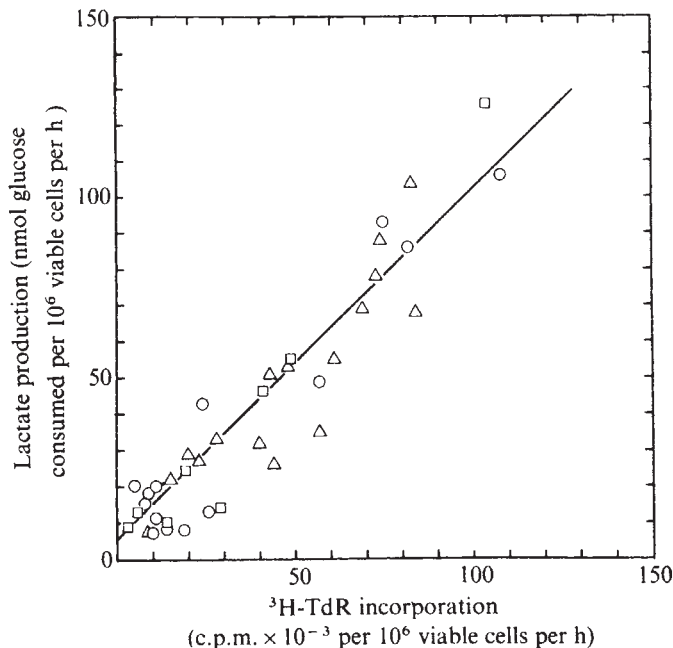


Fig. 2 Lactate production plotted against ^3H -TdR incorporation with data obtained from the assays described for Fig. 1. The data are from two experiments each with 1.25×10^6 cells per ml (□), 2.5×10^6 cells per ml (○) and 5×10^6 cells per ml (△). All time points from 24–72 h are presented. The line of best fit was obtained through least squares analysis of the data and $r = 0.93$.

revealed in Fig. 2. A plot of the rates of ^3H -thymidine incorporation against lactate production gave a straight line ($r=0.93$). But all cells can produce lactate when necessary and before a link between aerobic glycolysis and DNA synthesis can be accepted, explanations based on metabolic artefacts imposed by culture conditions must be considered. The hypothesis has been made that aerobic glycolysis appears as a result of the residence of cells in culture¹⁸. For the control lymphocytes in Fig. 1b, there was no significant increase in aerobic glycolysis during 120 h in culture. Even more convincingly, the high levels of glycolysis found in con A-stimulated cells did not persist with the cessation of DNA synthesis. Therefore, in the absence of proliferation, the metabolism of these cells did not shift towards aerobic glycolysis merely as an adaptation to culture conditions.

The data of Fig. 2 contribute to establishing the relationship between DNA synthesis and aerobic glycolysis in two ways. First, the determinations were done after 24–72 h in culture. Therefore, whether DNA synthesis was just beginning, at a maximum or at an end, a proportional amount of lactate was made. Second, these data are from experiments which used cultures of three different initial cell densities (1.25 , 2.5 or 5×10^6 cells per ml). Aerobic glycolysis does not seem to depend on cell density within this range, and the rate remains proportional to DNA synthesis.

Two further groups of experiments were carried out to eliminate other possible explanations for this phenomenon. In the first, the medium and serum were changed every 12 h to ensure the removal of toxic products and the presence of sufficient nutrients so that cellular metabolism would not be influenced. In further experiments culture pH was kept constant at either 6.9 with 15 mM PIPES or 7.7 with 10 mM HEPES. No alteration was seen in the relationship of DNA synthesis to lactate production in either experimental group.

These experiments show that aerobic glycolysis considerably increased together with DNA synthesis in con A-stimulated mouse spleen lymphocytes. Furthermore, with the return of these cells to the non-proliferative state, lactate production also fell. For these cells, aerobic glycolysis seems intrinsic to the proliferative response and is not dominated by culture conditions such as cell density, culture age, medium pH, exhaustion of nutrients or accumulation of toxic metabolites. Obviously, as other studies have shown, lactate production can be altered to a variable extent

by these factors^{12,13}. All cells will produce lactate anaerobically and glycolysis will increase when cell density begins to interfere with oxygenation. Similarly, when the glucose concentration is lowered to one tenth of physiological levels and one fourth of the concentration we found optimum for glycolysis by lymphocytes, lactate production by chick embryo fibroblasts will fall¹⁹. But when we tried to minimise the limitations imposed by the culture conditions, DNA synthesis and aerobic glycolysis seemed to be related. This is not to suggest that they are tightly coupled. Lactate production can and will change independently in varied conditions.

There are other possible explanations for our results. Small lymphocytes, although partially differentiated, are resting cells (G_0 phase) with sparse cytoplasm and a dense nucleus. Stimulation by con A promptly increased RNA and protein synthesis and enlarged the cytoplasm. Micrometric analysis showed that cell volume approximately doubled by the onset of DNA synthesis (our unpublished work). The many cellular changes which follow stimulation by con A presumably also include further differentiation. Con A bound to Sepharose beads has been shown to induce immunoglobulin production, a marker of cell differentiation, as well as cell division in mouse bone marrow lymphocytes²⁰. It is therefore possible that the increased aerobic glycolysis we observed is related to cell growth and/or differentiation rather than to DNA synthesis. We believe this is the less likely alternative. According to our results, the temporal correlation of lactate production is much better with DNA synthesis than with RNA and protein synthesis. This suggests that general cell growth is not the source. The process of cellular differentiation, however, does seem to take place together with cell division²¹. Immunoglobulin synthesis, for example, was greatest in late G_1 and early S phase in one study of synchronised lymphocytes²². In spite of the apparent dependence of differentiation on cell division, it seems that differentiation is not the stimulus for aerobic glycolysis. Once the differentiated state has been reached, lactate production rarely persists. But the most telling argument seems to be the clearly inverse relationship between aerobic glycolysis and the degree of differentiation of various hepatomas⁷. The final resolution of this question, however, awaits greater understanding of both cell division and differentiation.

We have proposed that the simultaneous decline in DNA synthesis and lactate production is important evidence that these two events are linked. It may reflect, however, a loss of viability of these cells. We believe this is not the case for several reasons. Our results are corrected for cell viability which remains stable from the peak of DNA synthesis (48–60 h) to 120 h. Cellular integrity can also be substantiated by the normal level of lymphocyte respiration observed 96 h after stimulation by con A (our unpublished work). But most important, the cell number continues to increase exponentially up to 96 h—a clear sign of viability.

It is true that the lymphocytes are unresponsive to con A after their proliferative response. We feel that this is to be expected. Stimulation by con A presumably produces the *in vitro* correlate of the final activation and differentiation which occurs *in vivo* in response to an antigenic challenge. It is unlikely that lymphocytes *in vivo* have the capacity for continuous division.

The question of whether or not the cancerous state increases aerobic glycolysis when growth rate is corrected for, has rarely been studied critically. Almost always controls have been normal undividing cells. It has been investigated, however, in chick embryo fibroblasts. In one study, transformation by Rous sarcoma virus did not result in enhanced lactate production⁹. A second group, however, found that if the ratio of aerobic glycolysis to respiration was determined, the ratio was increased by transformation¹³. Consequently, cancerous transformation may lead to a further shift of metabolism towards aerobic glycolysis but the evidence at this time is far from complete.

Our studies have also shown that glucose utilisation rises as expected during active glycolysis and, in addition, glucose transport increases approximately thirtyfold (unpublished work). Catabolism of glucose during this phase results predominantly in the formation of lactate and more than 95% of the label is

recovered as either lactate or glucose. At present, the reason for these alterations in glucose metabolism during DNA synthesis is unknown. Whatever the explanation proves to be, aerobic glycolysis may be a general characteristic of dividing cells. The Warburg effect, rather than a reflection of defective respiration, may prove to be a normal accompaniment of cell division.

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Further evidence for derepression of H-2 and Ia-like specificities of foreign haplotypes in mouse tumour cell lines

We reported earlier the appearance of specificities like those of foreign H-2 haplotypes on a mouse sarcoma after vaccinia virus infection¹. The presence of these foreign transplantation antigens was apparently sufficient to inhibit the growth of the tumour (unpublished data). Parallel work in man has shown qualitative changes in the HLA profile of fibroblasts grown in tissue culture and infected with SV40 virus². The mechanism by which a virus can induce changes in the pattern of cell surface histocompatibility antigens remains obscure.

We recently favoured a derepression hypothesis. According to this hypothesis, allelic differences in histocompatibility antigens are the products of closely linked genes³ most of which are repressed in normal circumstances. A virus or other agent could interfere with the repression system and cause derepression of certain genes and/or repression of others resulting in the appearance of allogeneic and/or loss of syngeneic specificities. In this report we present further data which are in line with this repression-derepression hypothesis. A spontaneous θ -positive mouse lymphoma (SL2) was found to express in addition to the H-2 specificities of the host's cells, alloantigens (H-2 and Ia) apparently of foreign haplotypes. A chemically induced lymphosarcoma (Gardner) on the other hand was found not to express one of the private H-2 specificities of the host's cells.

Tumour cells were propagated in ascites form in syngeneic recipients (SL2 in DBA/2 (ref. 4) (H-2^d) and Gardner in CBA/H (ref. 5) (H-2^k)). They were taken from the peritoneal cavity, tested in direct complement-dependent microtoxicity and also with a post-labelling assay measuring uptake of ¹⁴C-thymidine. The pattern of reaction of both tumours with a variety of sera of restricted

specificity, that is, alloantisera against H-2, non-H-2 (Thy 1.2 and Ly 4.2) and heteroantisera against B cells and mouse Ig is shown in Table 1. SL2 lymphoma cells reacted strongly with all antisera against private and public specificities of the host (haplotype: H-2^d; H-2 specificities: 4,31,3,8,13,28,35). Positive reactions against SL2 were also found with some other anti-H-2 sera which do not react with H-2^d lymphocytes and which are directed against private H-2 specificities of non-H-2^d haplotypes (that is H-2—2,17,19,20,30). Some of the extra reactivities against SL2 were further analysed by specific absorption procedures (Table 2). The cytotoxic effect of anti-H-2.1 and anti-H-2D.32 on SL2 was specifically removed with normal lymphoid cells from H-2.1 and H-2D.32 positive strains and could not be distinguished from the normal H-2 specificities present in the control tumour Gardner expressing H-2.1 and H-2D.32 in accordance with genetic expectation.

The results obtained with Gardner lymphoma show that a tumour may also lose certain H-2 specificities. Antiserum against the private H-2K specificity (H-2K.23) did not react with the lymphoma while it reacted with normal lymphocytes from the host. The absence of K.23 from the tumour cells was confirmed by absorption analysis and by indirect immunofluorescence. Another anti-H-2K^k serum, A.TL anti-A.AL was also negative in the cytotoxicity test. On the other hand, all the sera against public H-2 specificities of the H-2^k haplotype reacted strongly with the tumour and two sera (anti-H-2.16 and anti-H-2.35) gave strong extra reactions.

Ia specificities also seemed to be expressed on one of the Thy 1.2 positive tumours (see the reaction with A.TH anti-A.TL against SL2, Table 1). This was of particular interest in view of the fact that Ia antigens are expressed predominantly on B cells⁸ and macrophages^{9,10}. Several anti-Ia sera of restricted specificity (kindly provided by Dr Chella David) were titrated against SL2 tumour cells (Fig. 1). Some were strongly and others weakly positive, including a serum which was raised in an H-2^d hybrid mouse (A.TH × B10.Da) anti-A.TL. One serum (anti-Ia 6,7) was found to be negative. The cytotoxic anti-tumour activity of anti-Ia sera could be absorbed with spleen cells from mice of foreign H-2 haplotypes (that is B10.BR (H-2^k), B10.A (H-2ⁿ) and B10.A(4R) (H-2^h)) but not with spleen cells from H-2 identical mice (B10.D2 (H-2^d)) or from

Fig. 1 Cytotoxicity of anti-H-2D.4 (●), anti-Thy 1.2 (▲) and anti-Ia sera against SL2 tumour cells. A.TH anti-A.TL (○), anti-Ia 1,2,3,7,15,19; (A.CA × B10.HTT) anti-A.TL (△), anti-Ia 2,3,15,19; (A.BY × B10.HTT) anti-A.TL (□), anti-Ia 1,2,15,19; (A.TH × B10.D2) anti-A.TL (*), anti-Ia 1,2,3,19; (129 × B10A 4R) anti-B10A 2R (◆), anti-Ia 6,7.

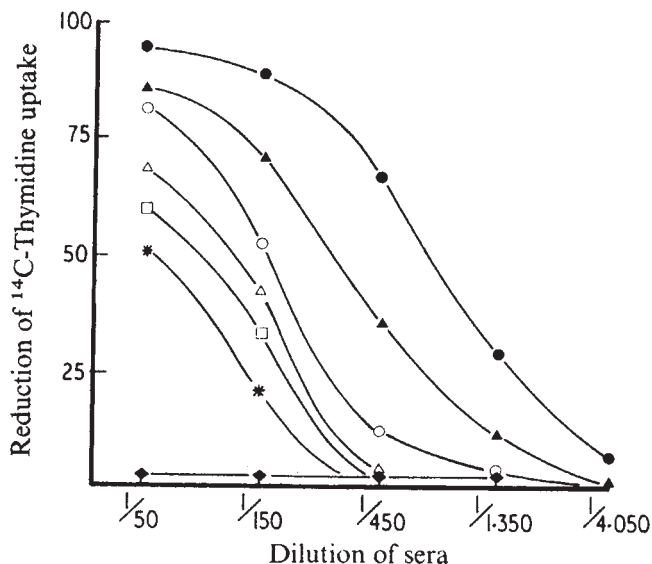


Table 1 Cytotoxic activity of anti-H-2 sera against SL2 leukaemia and Gardner lymphoma

Pretreatment of tumour cells with:		C'	SL2(H-2 ^d)		Reduction (%)	Gardner (H-2 ^k)		Reduction (%)
Sera	(Haplotype)		¹⁴ C-thymidine uptake	c.p.m. $\pm$ s.d.		¹⁴ C-thymidine uptake	c.p.m. $\pm$ s.d.	
—	—	—	15.792 $\pm$ 1.998			12.948 $\pm$ 642		
—	—	+	12.948 $\pm$ 399			13.539 $\pm$ 924		
Private								
Anti-H-2D.2	(b,g,h,j,)	+	7.119 $\pm$ 1.554		45	13.830 $\pm$ 1.920		0
Anti-H-2D.4	(a,d,i,t,u,y)	+	1.164 $\pm$ 102		91*	12.861 $\pm$ 954		5
Anti-H-2.9	(f)	+	12.429 $\pm$ 1.890		4	13.719 $\pm$ 1.329		0
Anti-H-2D.12	(s)	+	13.473 $\pm$ 360		0	13.131 $\pm$ 813		3
Anti-H-2K.15	(j)	+	3.366 $\pm$ 840		74	10.017 $\pm$ 1.095		26
Anti-H-2.16	(p)	+	5.826 $\pm$ 378		55	0.945 $\pm$ 144		93
Anti-H-2K.17	(q,y)	+	5.697 $\pm$ 546		56	12.996 $\pm$ 1.083		4
Anti-H-2.18	(r)	+	2.328 $\pm$ 222		82	9.882 $\pm$ 732		27
Anti-H-2K.19	(s,t)	+	3.495 $\pm$ 588		73	12.318 $\pm$ 1.527		9
Anti-H-2K.20	(u)	+	7.509 $\pm$ 1.035		42	13.590 $\pm$ 1.143		0
Anti-H-2K.23	(a,h,k,m)	+	14.310 $\pm$ 1.617		0	13.653 $\pm$ 888		0†
Anti-H-2D.30	(m,q,v)	+	4.788 $\pm$ 519		63	11.100 $\pm$ 1.362		18
Anti-H-2K.31	(d,g,o)	+	0.258 $\pm$ 72		98*	14.307 $\pm$ 1.146		0
Anti-H-2D.32	(k,o)	+	3.237 $\pm$ 630		75	0.945 $\pm$ 192		93†
Anti-H-2K.33	(b,i)	+	13.041 $\pm$ 1.353		0	11.370 $\pm$ 843		16
Public								
Anti-H-2.1		+	2.847 $\pm$ 306		78	1.488 $\pm$ 297		89†
Anti-H-2.3		+	0.387 $\pm$ 42		97*	0.540 $\pm$ 102		96†
Anti-H-2.5		+	14.418 $\pm$ 1.667		0	1.893 $\pm$ 243		86†
Anti-H-2.8		+	0.537 $\pm$ 204		96*	1.281 $\pm$ 72		91†
Anti-H-2.11		+	7.248 $\pm$ 48		44	2.571 $\pm$ 189		81†
Anti-H-2.13		+	0.399 $\pm$ 66		97*	14.349 $\pm$ 657		0
Anti-H-2.25		+	13.284 $\pm$ 369		0	7.581 $\pm$ 735		44†
Anti-H-2.28		+	0.645 $\pm$ 144		95*	13.890 $\pm$ 440		0
Anti-H-2.35		+	0.249 $\pm$ 27		98*	1.083 $\pm$ 219		92
Others								
Normal Mouse Serum		+	12.039 $\pm$ 993		7	12.996 $\pm$ 1.083		4
Anti-Thy 1.2		+	0.516 $\pm$ 54		96	1.146 $\pm$ 306		92
Anti-Ly 4.2		+	13.503 $\pm$ 1.824		0	13.608 $\pm$ 1.527		0
Anti-Ia (A.TH anti-A.TL)		+	0.906 $\pm$ 237		93	13.158 $\pm$ 651		3
CBA/H anti-BALB/c		+	0.654 $\pm$ 111		95	9.204 $\pm$ 630		32
BALB/c anti-CBA/H		+	7.770 $\pm$ 942		40	2.463 $\pm$ 354		82
Rabbit anti-MBLA		+	13.446 $\pm$ 1.440		0	7.446 $\pm$ 384		45
Rabbit anti-MIg		+	13.173 $\pm$ 1.899		0	13.818 $\pm$ 1.086		0

2 $\times$ 10⁴ Macrophage-depleted tumour cells (SL2 or Gardner) in 50 μ l of culture medium were incubated in round bottom wells of sterile microtitre plates with 1 μ l of antiserum for 1 h at 4 °C and then 30 min with 50 μ l of prediluted 1/10 rabbit complement at room temperature (C'). For SL2 the rabbit complement was previously absorbed with DBA/2 spleen and thymus cells for 1 h at 0 °C. After washing, 10 μ l of ¹⁴C-thymidine (4 μ Ci ml⁻¹) were added to the plates for a further 16 h. The results are expressed as the percentage reduction in thymidine uptake in test samples compared with control samples with complement and no antibody. Similar results were obtained by measuring ¹⁴C-uridine uptake.

For details of the H-2 alloantisera see NIH catalogue and supplements⁸. A.TH anti-A.TL serum was a gift of Dr Chella David, the other sera were raised and tested for specificity in the London Hospital Medical College.

*Expected positive reaction for an H-2^d cell: private specificities H-2D.4 and H-2K.31, public 3,8,13,28,35.

†Expected positive reactions for an H-2^k cell: private specificities H-2K.23 and H-2.D32, public 1,3,5,8,11,25 (ref. 7).

Table 2 Absorption of cytotoxic anti-tumour activity from anti-H-2 sera and anti-Thy 1.2 by normal lymphoid cells and brain tissue carrying the appropriate antigenic determinants

Antisera	Absorbing cells from*	C'	SL2 (H-2 ^d) ¹⁴ C-thymidine uptake c.p.m.± s.d.	Reduction (%)	Gardner (H-2 ^k) ¹⁴ C-thymidine uptake c.p.m.± s.d.	Reduction (%)
—	—	—	18.606± 942		12.945± 576	
—	—	+	14.535± 354		14.550± 1.134	
Anti-H-2 K.31	—	+	0.579± 117	96	14.601± 1.941	5
Anti-H-2 K.31	C57BL.10(31neg)	+	2.355± 54	84	15.540± 1.101	0
Anti-H-2 K.31	B10.BR(31neg)	+	1.281± 747	91	14.031± 522	4
Anti-H-2 K.31	B10.D2(31pos)	+	13.113± 2.016	11	14.706± 2.493	0
Anti-H-2.1	—	+	3.396± 1.107	77	3.957± 849	73
Anti-H-2.1	C57BL.10(1neg)	+	2.637± 726	82	5.829± 687	60
Anti-H-2.1	B10.D2(1neg)	+	6.546± 801	55	3.084± 450	79
Anti-H-2.1	B10.BR(1pos)	+	11.751± 882	19	12.108± 576	17
Anti-H-2D.32	—	+	4.233± 525	71	2.178± 423	85
Anti-H-2D.32	B10D2(32neg)	+	7.380± 1.284	49	6.813± 2.454	54
Anti-H-2D.32	B10.A(32neg)	+	3.054± 369	79	5.781± 1.794	60
Anti-H-2D.32	B10.BR(32pos)	+	12.273± 804	17	15.618± 783	0
Anti-Thy 1.2	—	+	1.368± 516	91	1.611± 213	89
Anti-Thy 1.2	CBA/H brain (Thy 1.2 pos)	+	15.390± 1.815	0	15.426± 1.575	0
Anti-Thy 1.2	AKR brain (Thy 1.2 neg)	+	3.426± 303	76	1.716± 531	88

*H-2 alloantisera prediluted 1/6 or 1/10 and anti-Thy 1.2 were absorbed with 5 $\times$ 10⁷ spleen cells from B10, B10.BR, B10.D2, B10.A mice or with brain tissue from CBA and AKR mice respectively for 1 h at 4 °C and tested back against SL2 (H-2^d) and the control tumour Gardner (H-2^k). The results are expressed as % of reduction in ¹⁴C-thymidine uptake in test samples as compared with control samples with complement (C') and no antibody.

Table 3 Absorption of cytotoxic anti-tumour activity against SL2 leukaemia from anti-Ia sera by normal lymphoid cells carrying the appropriate Ia determinants

Antisera	Absorbing cells from*	Potential anti-Ia activity	C'	¹⁴ C-thymidine uptake c.p.m. $\pm$ s.d.	Reduction (%)
—	—	—	—	10.158 $\pm$ 333	—
(A.CA $\times$ B10.HTT) anti-A.TL	—	2, 3, 15, 19	+	8.505 $\pm$ 312	—
(A.CA $\times$ B10.HTT) anti-A.TL	B10.HTT (H-2 ¹³)	2, 3, 15, 19	+	2.466 $\pm$ 126	71
(A.CA $\times$ B10.HTT) anti-A.TL	C57BL/10(H-2 ^b)	2, 15, 19	+	3.168 $\pm$ 384	63
(A.CA $\times$ B10.HTT) anti-A.TL	B10.BR (H-2 ^k)	—	+	3.810 $\pm$ 132	55
(A.CA $\times$ B10.HTT) anti-A.TL	B10.D2 (H-2 ^d)	2, 3, 19	+	6.408 $\pm$ 675	25
A.TH anti-A.TL	—	1, 2, 3, 7, 15, 19	+	3.996 $\pm$ 843	53
A.TH anti-A.TL	A.TH (H-2 ¹²)	1, 2, 3, 7, 15, 19	+	0.835 $\pm$ 252	90
A.TH anti-A.TL	B10.A(4R) (H-2 ^{h4})	7, 15, 19	+	2.410 $\pm$ 513	72
A.TH anti-A.TL	B10.A (H-2 ^a)	—	+	6.578 $\pm$ 704	22
A.TH anti-A.TL	—	—	+	6.431 $\pm$ 750	25

*60 μ l of 1/6 or 1/10 diluted antiserum was absorbed with 5×10^7 spleen cells from B10, B10.D2, B10.BR, B10.A, B10.A(4R), B10.HTT or A.TH mice for 1 h at 4 °C and tested back against SL2 cells in presence of complement. Tumour cells were postlabelled with ¹⁴C-thymidine overnight. Results are expressed as % or reduction in ¹⁴C-thymidine uptake compared with complement alone (C'). Ia specificities in H-2^d haplotypes 6,7,8,11,15,16 (ref. 11).

serum donor strains (B10.HTT, A.TH) (Table 3). All data taken together suggest that the SL2 lymphoma expresses Ia specificities of foreign haplotypes (most likely Ia 1,2,3). Of particular biological importance now will be to establish whether the H-2- and Ia-like specificities of foreign haplotypes on SL2 are the tumour specific transplantation antigens which have already been isolated and partially purified from these tumour cells¹².

A theoretical possibility is that a virus on the surface of a cell and in close proximity to a histocompatibility antigen could produce a new antigen pattern which resembles a foreign transplantation antigen. If this were the explanation we should expect anti virus activity to be removed by absorption with normal lymphoid cells and the normal H-2 configuration to be altered^{13,14}. We could find no alteration of the normal H-2^d profile, nor was it possible to remove anti-virus activity with normal lymphoid cells. The same NIH reference sera which caused anomalous anti-tumour reactions in Nowinski's and Klein's studies¹⁵ (D-4 and D-31) reacted normally with all our tumour cells (F.G., V.S. and H.F., unpublished). Furthermore, it is unlikely that extra reactions are due to "cross-reacting antigens" in damaged cells because our assay measures only metabolic activity in healthy cells.

From our specific absorption data we favour the above-mentioned derepression hypothesis, although we cannot formally exclude the possibility of activated endogenous virus directly creating new H-2-like determinants on the tumour cell surface. A derepression mechanism would imply that every member of a species carries a full set of structural genes for all the different 'alleles' of a particular system. Since gene repression is much more extensive than gene expression, it would be surprising if the species genes were not widely distributed. Several recent findings which could be explained by derepression of structural genes, normally silent, are: (1) the appearance of an unexpected human allotype in lymphoid tumour lines transferred into hamsters¹⁶, (2) the transient and unpredictable expression of IgG^a allotype in an IgG^b mouse¹⁷, (3) expression of allotypes of the "wrong kind" in rabbits after immunisation with *Micrococcus lysodeikticus*¹⁸, (4) the appearance and disappearance of HLA antigens in cultured lymphoblastoid cell lines^{19,20} and (5) variations of the expression of HLA antigens on human fibroblasts *in vitro*²¹.

If there are depressed H-2-like antigens one would expect to find also examples of repression. This could be an explanation for the loss of the K^k private specificity of Gardner lymphoma. Experiments are under way to find out whether the cells could be activated (derepressed) to produce the missing H-2K^k antigen.

Whatever the precise mechanism of the production of the observed extra specificities, the fact that they were serologically indistinguishable from major histocompatibility

complex (H-2 and Ia) specificities, opens a new conceptual way of looking at the question of surveillance of tumours and transplants.

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Release and synthesis of prolactin by rat pituitary cell strains are regulated independently by thyrotropin-releasing hormone

THYROTROPIN-releasing hormone (TRH) is the tripeptide pyro-glutamyl-histidyl-proline-NH₂, which causes the release of prolactin and thyrotropin from the anterior pituitary gland in

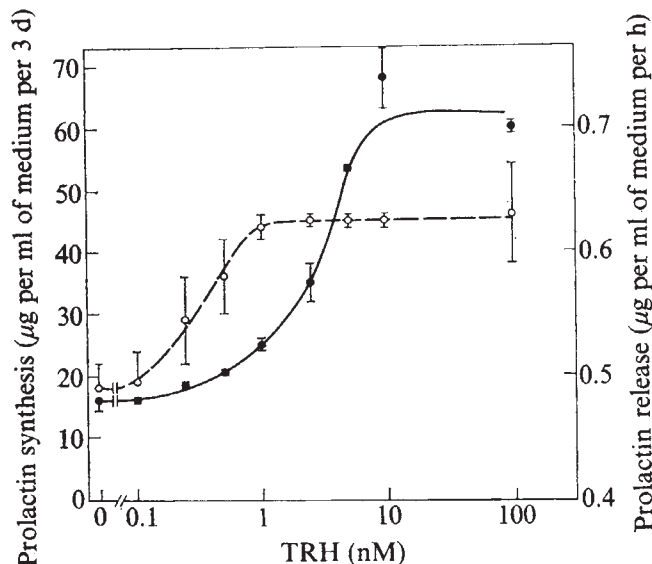


Fig. 1 Effects of increasing concentrations of TRH on prolactin synthesis (●) and release (○). GH cells were plated and grown to about 0.5 mg protein per dish in Ham's F10 medium (Gibco) supplemented with 15% horse serum and 2.5% foetal calf serum⁸. All the experiments described here were then performed in serum-free medium as follows. Three to four days after plating, the medium was removed, the plates were rinsed with 1 ml of Newman and Tytell serum-free medium (Gibco) and then 2.5 ml of this medium were added to each 60-mm culture dish. Solutions of TRH or analogues were added in 0.1 ml of sterile 0.15 M NaCl to give the indicated final concentrations of TRH. At the end of 1 h, medium was collected for determination of prolactin release; fresh medium was added and prolactin synthesis was determined 3 d later. Prolactin was measured by microcomplement fixation⁶. The cells do not store much prolactin; therefore measurements made at 3 d reflect almost entirely (99%) hormone synthesis⁷. In the dose-response curves here and in Fig. 2, each point represents the mean of determinations from duplicate dishes; vertical bars give the ranges; ○, release; ●, synthesis.

man and other animals¹⁻³. Although the isolation of TRH was based on its ability to release thyrotropin, injection of TRH usually releases both prolactin and thyrotropin^{4,5}. In spite of this knowledge, the physiological role of TRH in the control of either thyrotropin or prolactin secretion is not clear. We report here that the release and synthesis of prolactin are controlled independently by TRH in rat GH cells.

GH cells are clonal strains of rat pituitary cells in culture which synthesise and secrete prolactin and growth hormone, but not thyrotropin or other anterior pituitary hormones⁶. TRH stimulates the release of prolactin from GH cells within minutes; later, 3-4 h after addition of TRH to the medium, the synthesis of prolactin begins to increase and reaches a maximum 24-48 h later⁷.

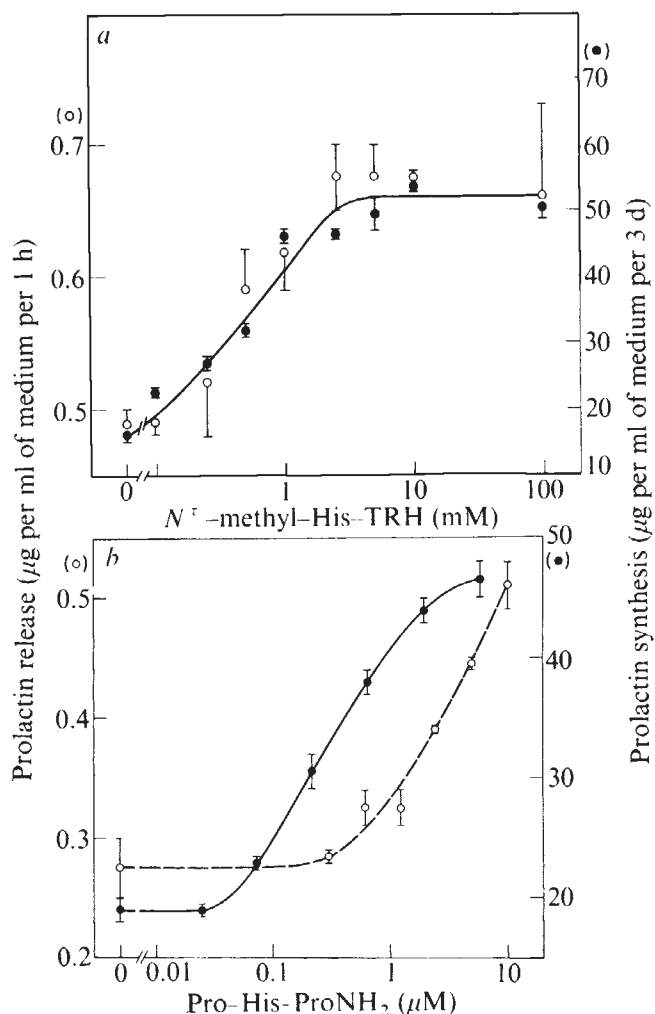
Dose-response curves for the TRH-induced increases in prolactin release and synthesis are shown in Fig. 1. Half-maximal stimulation of prolactin release occurred at 0.3 nM TRH; the half-maximal increase in synthesis occurred at 3 nM. Three possible explanations for this difference are as follows. (1) TRH is not stable in the medium and therefore the concentration of peptide is decreasing during a 3-d synthesis experiment. (2) Fewer TRH receptors need to be occupied to stimulate prolactin release than to stimulate its synthesis. The binding of TRH to GH cells has been measured; there is half-maximal binding at 11 nM (ref. 9). Because half-maximal stimulation of prolactin synthesis occurs at 3 nM, it seems that not all receptors need to be occupied to stimulate synthesis, and occupancy of even fewer may be required to cause release. (3) The hormone-receptor interaction that mediates prolactin release is different from that which mediates synthesis. We have investigated these possibilities.

TRH that is bound to GH cells is degraded¹⁰; however, after

3 d this amounts to less than 1% of the TRH present in the medium, a finding which cannot account for the tenfold difference between TRH concentrations required for release and synthesis. Although TRH is degraded in whole serum^{11,12} we have shown that the peptide is stable in the conditions of these experiments in serum-free medium. The serological activity of TRH that had been incubated with GH cells for 3 d was the same as that of unincubated TRH with an antiserum to TRH which does not react with pGlu-His-Pro, one of the major degradation products of TRH (unpublished results). In addition, the biological activity of TRH is not lost after incubation with the cells. TRH (300 nM) was incubated with GH cells for 3 d and then aliquots of this medium were tested for prolactin synthesis-stimulating activity by incubation with fresh cells for a further 3 d. Half-maximal stimulation by TRH which had been incubated with GH cells occurred at 3.7 and 1.6 nM in two separate experiments, which is in agreement with the value of 3 nM (Fig. 1) for unincubated TRH.

If the second possibility were true, that is that fewer receptors need to be occupied to stimulate prolactin synthesis than to stimulate release, then only one receptor would be involved, and any active analogue should cause release at lower concentrations than synthesis. Data in Fig. 2 reveal that this is not true. An analogue of TRH (Fig. 2a) with a methyl group on the third

Fig. 2 Effects of increasing concentrations of two analogues of TRH on prolactin release and synthesis. *a*, Effects of pyroglutamyl-*N*⁷-methyl-histidyl-proline-NH₂; *b*, prolyl-histidyl-proline-NH₂. Other experiments showed that a maximum increase in synthesis had been reached with the highest concentration of prolyl-histidyl-proline-NH₂ that is shown. Experimental procedures were as described in the legend to Fig. 1. ●, Synthesis; ○, release.



nitrogen of the imidazole ring of the histidine (*N* τ -methyl-histidyl-TRH) caused half-maximal stimulation of release and synthesis at the same concentration, about 0.5 nM. Another analogue (Fig. 2b) with a proline residue substituted for the pyroglutamyl ring was more active in stimulating synthesis than release, so that half-maximal stimulation of synthesis (0.25 μ M) was reached before there was any detectable increase in release. This finding demonstrates that increased release of prolactin is not necessary to trigger increased synthesis, and that synthesis can be stimulated independently.

Table 1 summarises the results for the analogues of TRH that we have tested. Variations shown in the pyroglutamyl and histidyl residues all influenced release more than they did synthesis, resulting in ratios of synthesis to release of less than 10 compared with a ratio of 10 for TRH. Modifications in the C-terminal proline amide residue in most cases seemed to affect release and synthesis to similar extents, for with these analogues the ratios of synthesis to release remained about 10 or greater. One analogue, pGlu-His-ProNH(CH₂)₆NH₂, did not fall into this category. It may be that this peptide is not stable and is metabolised into a more active compound; studies are in progress to test the reason for this discrepancy.

These data are the first evidence of which we are aware that hormone release and synthesis can be influenced separately by a single regulatory factor.

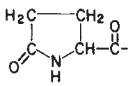
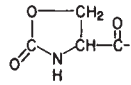
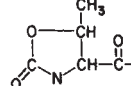
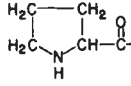
Since prolactin release and synthesis can be affected differently by analogues of TRH, the pathways through which these two processes are mediated must be separate. The experiments performed so far have involved agonists of TRH; the different

responses to different agonists indicate that there are two separate interactions, but they do not prove there are two physically distinct sites. For example, TRH might bind to one site, activating adenylate cyclase to enhance release, while causing aggregation of receptors to stimulate synthesis: modifications in the structure of TRH could influence one process and not the other. An antagonist of TRH that would be capable of interfering with one process and not the other would demonstrate that the two sites are physically separate.

The TRH receptor in GH cells has been characterised and only one class of high-affinity sites has been detected; there is half-maximal saturation of these sites at 11 nM compared with 2–3 nM for the half-maximal increase in prolactin synthesis⁹. The *N* τ -methyl-histidyl analogue competes with ³H-TRH for binding more effectively than does TRH itself¹³. The affinity of the receptor and the specificity of the methyl derivative indicate that the characterised receptor has the properties that would be expected if it were involved in synthesis and not release. If a separate receptor for release exists, it must therefore be present in smaller numbers than the synthesis receptor.

Some analogues of TRH used in this study have also been tested for their effects on the release of thyrotropin in intact animals and from isolated pituitary glands. When data from this report on prolactin release are compared with data on thyrotropin release from normal pituitary glands, the relative activities of most analogues compared with TRH are very similar^{14,15}, this is also true in one report in which release of both prolactin and thyrotropin was measured, using several TRH analogues, in rats³. These findings suggest that the

Table 1 Half-maximal concentrations of TRH and analogues of TRH required for stimulation of prolactin release and synthesis

Peptide	Release [peptide] for half maximum (nM)	Synthesis [peptide] for half maximum (nM)	[Peptide] for synthesis [Peptide] for release
X-His-ProNH ₂			
TRH			
	0.3	3	10
	1	3.5	3.5
	5	3.5	0.7
	1,250	250	0.2
pGlu-X-ProNH ₂			
pGlu- <i>N</i> τ -MeHis-ProNH ₂	0.5	0.5	1
pGlu- <i>d</i> His-ProNH ₂	25	100	4
pGlu-Lys-ProNH ₂	> 2,000	> 2,000	—
pGlu-Arg-ProNH ₂	> 2,000	> 2,000	—
pGlu-His-X			
pGlu-His-Pro	200	4,000	20
pGlu-His-ProNHCH ₃	4	50	12
pGlu-His- <i>d</i> ProNH ₂	300	3,000	10
pGlu-His-isoaminobutyric acid	300	3,000	10
pGlu-His-ProNH(CH ₂) ₆ NH ₂	300	20	0.07
pGlu-His-ProN(CH ₃) ₂	> 1,000	> 1,000	—

Effects of each analogue on prolactin release and synthesis were measured in complete dose-response experiments such as those shown in Figs 1 and 2. These values are the result of at least two dose-response curves; the half-maximal values in separate experiments did not differ more than twofold.

receptor mediating thyrotropin release is similar to the receptor mediating prolactin release. But one example in which the response to an analogue is not similar for prolactin and thyrotropin is the $N\tau$ -methyl-histidyl analogue which is eight times more active than TRH in stimulating thyrotropin release in intact animals¹⁴, but is not more active than TRH in causing prolactin release from GH cells. The effect of the $N\tau$ -methyl-histidyl analogue on prolactin release in intact animals has not yet been reported. If GH cells are a valid model system for the normal pituitary gland, this derivative should not be more active than TRH in releasing prolactin in the normal pituitary gland. When further comparisons are made, other differences between prolactin release and TSH release may be discovered.

TRH has been reported to affect the central nervous system, for example, enhancing behavioural excitation produced by a combination of tranlycypromine and L-tryptophan¹⁶, and causing rapid hypothermia when injected intraventricularly in the cat¹⁷. TRH inhibited the firing of some neurones of the hypothalamus when applied microiontophoretically¹⁸, and it has been suggested as a neurotransmitter¹⁹. It therefore may not be surprising that TRH, like other established neurotransmitters, has more than one receptor.

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Immunofluorescence labelling of gonadotropin receptors in enzyme-dispersed interstitial cells

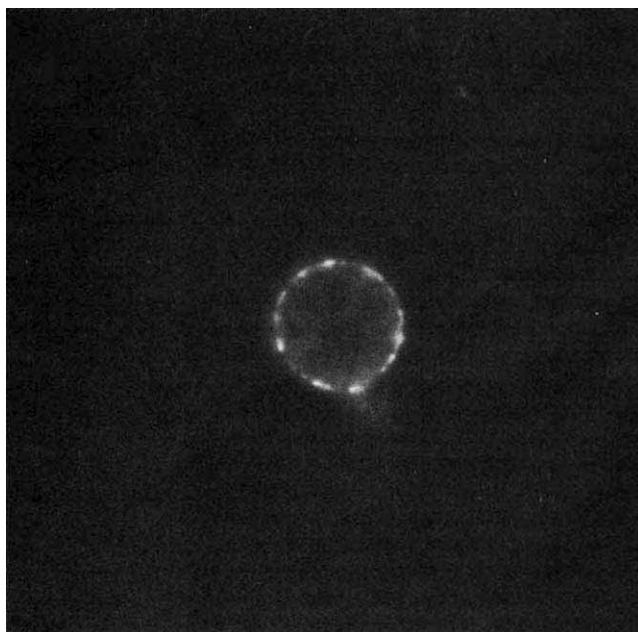
PEPTIDE hormone receptors in target tissues have been extensively analysed *in vitro* by binding studies with radio-active hormone derivatives. Although these methods have been of great value for characterisation of the binding sites for peptide hormones, there is also need for methods to examine the localisation and distribution of hormone receptors in isolated target cells. For this purpose, we have used an immunofluorescence technique to label the luteinising hormone (LH) receptors of the interstitial cells of the rat testis. This method has demonstrated that LH receptors are distributed as patches on the cell surface, and are rapidly lost from about 50% of the labelled cells after hypophysectomy. Also, occupancy of receptors by endogenous LH was found to persist for several days in a significant proportion of the interstitial cells.

Specific receptor sites for LH were originally identified in the interstitial cells of the testis by autoradiographic and immunohistological procedures^{1,2}. LH receptors have also been characterised *in vitro* by binding studies with radioiodinated LH or human chorionic gonadotropin (hCG) in testis homogenates and dispersed interstitial cells^{3–6}. The gonadotropin receptors of the rat testis have been shown to be associated with the plasma membrane of the interstitial cell during sucrose density gradient centrifugation and hormone binding studies⁷. In addition, about 50% of enzyme-dispersed testicular interstitial cells have been shown to possess steroidogenic enzyme activity characteristic of Leydig cells, the LH-responsive androgen-secreting cells of the interstitium⁸. The localisation and distribution of LH receptors in isolated interstitial cells has now been examined by an indirect immunofluorescent method using high titre rabbit antiserum to hCG and fluorescein-conjugated goat anti-serum to rabbit 7S globulin, after exposure of the cells to a saturating concentration of hCG.

Interstitial cell suspensions were prepared from decapsulated testes of mature Sprague–Dawley rats by collagenase dispersion as described previously^{6–8}; all incubation and washing steps being carried out in medium 199 containing 0.1% BSA. To saturate the LH receptors with gonadotropin, 0.5-ml aliquots of the cell suspension containing 5×10^6 – 10×10^6 cells ml⁻¹ were incubated in plastic centrifuge tubes with 100 IU hCG (Pregnyl, Organon) at 34 °C for 30 min. Cells were then washed twice with 40 ml medium, sedimented at 500g for 10 min, and resuspended in 0.6 ml medium before incubation at 34 °C for 30 min with rabbit anti-hCG serum at a final dilution of 1:60. The titre of the antiserum, in terms of the dilution required to bind 50% of a tracer quantity (50 pg) of ¹²⁵I-labelled hCG (100 μ Ci μ g⁻¹), was approximately 1:10⁶. The cells were then washed four times with 40 ml medium and resuspended in 0.3 ml medium, containing 5 μ l fluorescein-conjugated goat antiserum to rabbit 7S γ -globulin (Hyland, California; fluorescein–protein molar ratio, 3.6; 15.0 mg total protein ml⁻¹). After incubation for 30 min at 4 °C, the cells were washed twice with 40 ml medium and examined under a Leitz epi-illuminated fluorescence microscope.

In preparations obtained from intact rats, 50% of the

Fig. 1 Immunofluorescent labelling of collagenase-dispersed interstitial cells from intact rat testis, showing bright speckling around the circumference of the cell ($\times 1,350$).



interstitial cells exhibited marked fluorescence. This proportion corresponds to that previously observed to possess 3β hydroxysteroid dehydrogenase activity on histochemical analysis of enzyme-dispersed interstitial cells from the rat testis⁸, and represents the Leydig cell population of the testis interstitium cells. When such cells were examined by focusing on the equatorial plane, a brightly speckled band of fluorescent spots was visible around the circumference of the cell (Fig. 1). Adjustment of the depth of the visual field revealed discrete fluorescent areas over the entire cell surface. In most cells, aggregates of fluorescent spots could also be seen, but no polarisation of the fluorescent patches was observed.

At least 100 cells were counted from each cell preparation by two independent observers. Of the control cells, $10 \pm 2\%$ (s.e.) exhibited positive fluorescence when anti-hCG serum was replaced by normal rabbit serum or antiserum to angiotensin I. Such control preparations were evaluated with each experimental group, and the number of non-specifically labelled cells was subtracted from the total positive cell count. As shown in Fig. 2, approximately 40% of the interstitial cells from intact animals exhibited specific positive fluorescence. In these animals, significant fluorescent labelling could also be demonstrated in a high proportion (about 30%) of cells which had not been saturated with hCG before immunofluorescent labelling. This suggested that hCG antibodies could be taken up by endogenous LH bound to receptor sites on the cell surface. The ability of the hCG antiserum to react with rat LH was confirmed by the demonstration of direct binding of ^{125}I -labelled rat LH, and by measurement of the binding affinities for ^{125}I -labelled hCG (10^{11} mol^{-1}) and rat LH (about 10^8 mol^{-1}). Although the affinity of the antiserum for rat LH was considerably lower than the affinity for hCG, the combination of moderate affinity for rat LH and extremely high titre was adequate to account for the observed binding of antibody to endogenous LH present on the cell surface.

Immunofluorescence labelling of LH receptors was also carried out on interstitial cells obtained from mature animals at various intervals after hypophysectomy. Such cell preparations showed a significant decrease in fluorescent labelling carried out with or without saturation by hCG during the first incubation. The percentage of fluorescent cells in hCG-treated preparations decreased by 50% on day 6 after hypophysectomy, and remained constant at this level for up to 18 d. The initial fall in receptor sites, and the relatively constant level thereafter, are consistent with previous observations which demonstrated that hypophysectomy was followed by a decline in binding capacity within the first 3 d of operation, and a subsequent plateau at 30–50% of the control level^{7,9}.

Our observations suggest that hypophysectomy is followed by a rapid loss of receptor sites in about 50% of the Leydig cells in the testis interstitial cell fraction, rather than by a more generalised decrease in the number of receptor sites per cell. In the latter case, a moderate decrease in immunofluorescence would be observed in all cells, rather than the loss of a fraction of the labelled cell population which occurred after hypophysectomy. These findings suggest that a pituitary factor, possibly LH itself or prolactin, is necessary for maintenance of the LH receptors in a proportion of the Leydig cell population. In other Leydig cells, gonadotropin receptor sites are maintained for more prolonged periods after hypophysectomy, and represent a more stable population which is relatively independent of pituitary control.

In cell preparations not pretreated with hCG, the initially high level of fluorescence is attributable to occupancy of receptors by endogenous LH. Such fluorescence decreased in a serial fashion after hypophysectomy, but did not become undetectable until almost 2 weeks had elapsed. Prolonged binding of endogenous gonadotropin to receptor sites

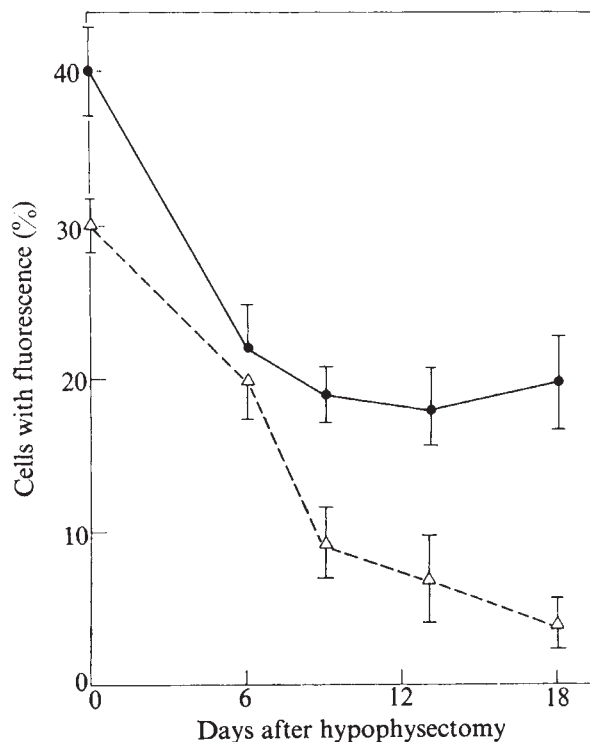


Fig. 2 Quantitative changes in immunofluorescence labelling of collagenase-dispersed interstitial cell preparations from mature male rats during the 3 weeks after hypophysectomy. Immunofluorescence studies were carried out on cells preincubated with (●) or without (△) 100 IU hCG. Each point represents the mean $\pm$ s.e. of six determinations.

is an unexpected finding in view of the general belief that hormone and drug actions are terminated when receptor sites are vacated by the regulatory ligand. Previous *in vitro* studies with labelled gonadotropins indicated that dissociation of hCG from testicular receptors is probably an extremely slow process^{4,10,11}. Our demonstration of prolonged occupancy by endogenous gonadotropins suggests that dissociation of the hormone–receptor complex also proceeds slowly *in vivo*, and that occupied receptors may be rendered inaccessible to further hormone stimulation for a prolonged period. A final speculation to be drawn from these studies is that the receptors for gonadotropin may function only once, and that occupied receptors could be inactivated or degraded, rather than reutilised during future hormonal stimulation. The use of immunofluorescent methods for detection of peptide hormone receptors in isolated target cells has considerable potential for investigation of the turnover, regulation and organisation of receptors within the cell membrane.

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Effect of testosterone, sex and age on experimentally induced arterial thrombosis

THE widespread use of large doses of oestrogenic steroids as contraceptive agents or for maintenance therapy in post-menopausal women has led to damaging effects on the cardiovascular system. Most of the reports relate to the increased thromboembolic phenomena which have been observed in both women and men treated with female hormones^{1,2}. In sharp contrast there are no data on the effect of androgenic steroids on the development of arterial thrombosis, in spite of the fact that the incidence of ischaemic heart disease in human males is much greater than in the pre-menopausal females³.

Hornstra and Vendelmans-Starrenburg⁴ have described an ingenious method for the induction of experimental arterial occlusive thrombi in rat. The method results in 100% incidence of thrombosis and yields highly reproducible results. Histological analysis of the thrombi showed that their structure and composition is comparable to arterial thrombi found in humans. We used this technique to test the effect of testosterone or oestradiol on thrombus formation in rats of different age groups since the incidence of primary thrombosis (ischaemic heart disease and cerebrovascular accidents) is known to increase with age in both males and females⁵. A polyethylene loop-shaped cannula was inserted in the abdominal aorta of 229 Wistar rats of both sexes aged 1.5, 3, 4, 6 or 12 months. The mortality rate, dry thrombus weight and obstruction time were used as criteria for the degree of the development of occlusive arterial thrombosis.

This phenomenon was found to be significantly more manifested in male than in female rats which were subjected to the same endothelial trauma (Figs 1 and 2). The mortality rate of animals 3, 4, or 6 months of age was approximately twice as high in male rats as in females ($P < 0.01$). This rate increased

with the age of the animal (Fig. 1). Twelve-month-old male rats had the highest mortality (52.2%). Female rats at the same age also had a high mortality rate (42.1%), but it was not significantly different from that of the males ($P > 0.05$). The only exception to this observation was in 45-d-old rats, where the mortality rate of these younger animals was lower than in the older rats; no significant difference in mortality was detected between males (4.6%) and females (4.3%) (Fig. 1).

Thrombus size was determined in animals killed 5 d after the aorta loops had been inserted. The excised aorta was perfused with saline, opened longitudinally and the thrombus removed. After drying over silica gel *in vacuo* for 24 h, the thrombus material was weighed. No measurable thrombus formation was observed in young rats of either sex at the age of 45 d. Statistically significant differences were found in the size of the thrombi between male and female rats at the age of 3, 4 and 6 months ($P < 0.01$) (Fig. 1). In 6-month-old animals this difference was most pronounced. After this age both males and females became increasingly responsive to the damage inflicted, and the differences between the males and females were no longer significant ($P > 0.05$). We observed no relationship between the thrombus and the body weight of the animals in the same group.

The obstruction time was determined by observing the colour of the blood which flows through the part of the cannula that projects out of the body. The obstruction time decreased progressively in males as well as in females at 3, 4, 6 and 12 months (Fig. 2). There were statistically significant differences ($P > 0.01$) in the obstruction time of male and female rats at the age of 3, 4 and 6 months. Again, sex differences were no longer apparent at 12 months (Fig. 2). During the experimental period (5 d) no obstruction of the loops occurred in animals of both sexes at the age of 45 d.

Species differences may be significant in the incidence of thrombus formation in the two sexes. In consequence, arterial thrombosis in 27 male and female New Zealand rabbits aged 6 months was studied using the method of Maekawa *et al.*⁶. Thrombus formation in the femoral artery was observed in 90.9% of male rabbits, whereas the occurrence of thrombus was established in 56.2% of females ($P < 0.01$).

Testosterone and oestradiol effects were studied in another series of experiments, using 194 Wistar male and female rats, aged 3 months. Depo-testosterone cypionate (Upjohn; 10 mg kg⁻¹) was injected subcutaneously twice a week for four consecutive weeks. Depo-oestradiol cypionate (Upjohn) was administered subcutaneously at a dose of 100 µg kg⁻¹ with the same schedule. Control animals of both sexes received cottonseed oil (0.1 ml subcutaneously). Occlusive arterial thrombosis was induced and the mortality rate, dry thrombus weight and obstruction time were determined. The mortality rate in the

Fig. 1 Thrombus weight ($\pm$ s.e.m.) and mortality rate of normal male (open columns) and female rats (hatched) at different ages.

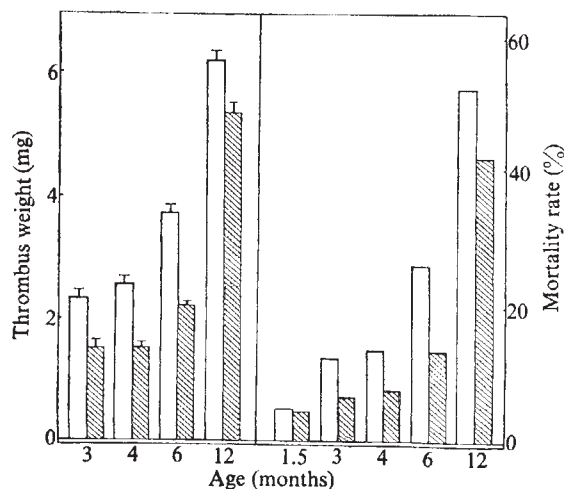


Table 1 Effect of testosterone, oestradiol and Flutamide on mortality rate of rats with occlusive arterial thrombosis

Group	Sex	N	Mortality rate (%)	Significance
Control	Male	28	14.3	
	Female	27	7.4	
Testosterone	Male	48	62.5	$P < 0.001^*$
	Female	35	34.3	$P < 0.02^*$
Oestradiol	Male	24	8.3	$P > 0.05^*$
	Female	32	6.3	$P > 0.05^*$
Flutamide + testosterone	Male	30	33.3	$P < 0.02^\dagger$
	Female	42	16.7	$P < 0.05^\dagger$

Depo-testosterone cypionate (10 mg per kg body weight) was injected subcutaneously twice weekly for 4 consecutive weeks. Depo-oestradiol cypionate (100 µg per kg body weight) was administered subcutaneously on the same schedule as testosterone. Flutamide (50 mg per kg body weight) was injected daily subcutaneously for 2 weeks. Control animals received cottonseed oil (0.1 ml subcutaneously).

*Compared with control rats of the corresponding sex.

†Compared with testosterone-treated rats of the corresponding sex.

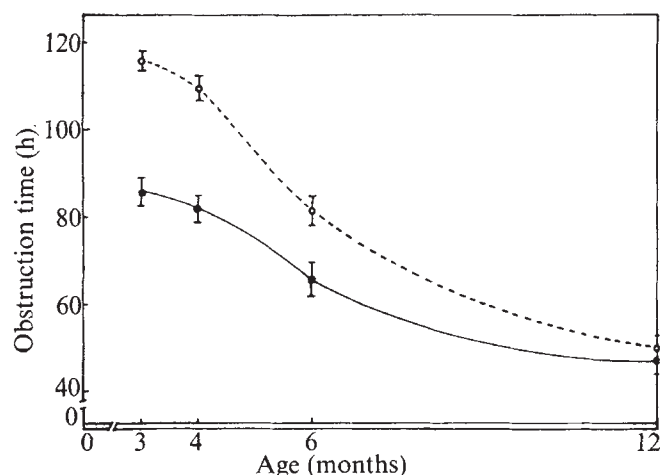


Fig. 2 Obstruction time ($\pm$ s.e.m.) in rats of different age. ●, Males; ○, females.

control groups, injected with cottonseed oil, was comparable with those of 4-month-old untreated rats (Fig. 1 and Table 1). Testosterone treatment increased the mortality rate more than fourfold in male as well as in female rats (Table 1). Results from oestradiol treatment showed a tendency toward a decrease in the mortality in both sexes; however, this effect was not statistically significant ($P > 0.05$).

The values for thrombus weight and obstruction time of the 4-month-old rats from our earlier experiments were comparable with those of the controls injected with cottonseed oil (Figs 1, 2 and 3). Thrombus weight in male rats treated with testosterone was markedly increased ($P < 0.001$) reaching an overall maximum of 12.10 ± 0.45 mg. Testosterone treatment of the female animals increased thrombus weight about threefold ($P < 0.01$) (Fig. 3). Oestradiol decreased significantly ($P < 0.05$) the thrombus weight in male rats only (Fig. 3), but had no significant effect in the females.

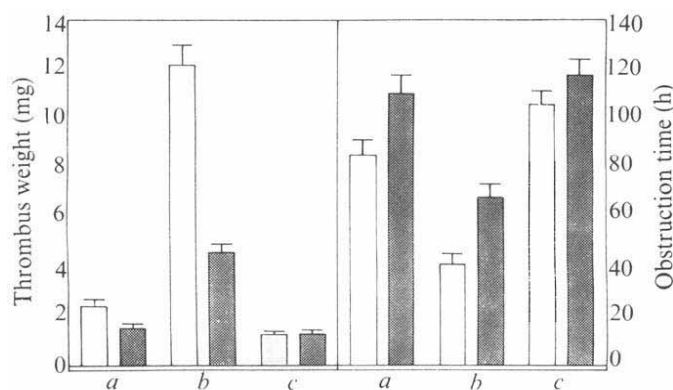


Fig. 3 Thrombus weight and obstruction time of testosterone-(b) and oestradiol-treated rats (c), compared with control (a) male (open columns) and female rats (solid). $\pm$ s.e.m. indicated.

Testosterone treatment shortened the obstruction time in both male and female rats ($P < 0.01$). Oestradiol increased significantly ($P < 0.05$) the obstruction time of male rats only, but did not show an appreciable effect in females (Fig. 3).

Flutamide (α - α -tri-fluoro-2-methyl-4-nitro-*m*-propionolide; Schering) a non-steroidal anti-androgen was tested together with testosterone in animals of both sexes. Occlusive arterial thrombosis was induced in male and female rats treated with a combination of Depo-testosterone cypionate 10 mg per kg body weight subcutaneously twice a week, and Flutamide 50 mg per kg body weight subcutaneously daily for

2 weeks. The anti-androgen significantly decreased the mortality rate of both male and female rats when treated with testosterone alone (Table 1).

These data indicate that there is a significant sex difference in thrombus size, obstruction time and mortality rate in rats with occlusive arterial thrombosis, males having approximately twice the thrombus size and death rate compared with females; thrombus weight and mortality rate increases with age; pronounced sex differences also exist in the susceptibility to arterial thrombi formation in rabbits; sex differences in thrombus size, obstruction time and mortality rate in rats are exacerbated by injection of testosterone but not by oestradiol; and the androgen effect in the development of arterial thrombosis seems to be specific since the anti-androgen, Flutamide, significantly reduced the mortality rate in rats treated with testosterone.

These data are consistent with the observation of Amos *et al.*⁹ that the amount of dog serum required to produce venous thrombosis in rats is 10–20 times higher for female than for male recipients, and that testosterone treatment of female rats reduced the required dose of serum to that of the male.

The enhanced arterial thrombi formation in male rats is also consistent with the much greater observed⁷ ability of platelets to aggregate in male than in female rats and guinea pigs. Similarly, the exacerbating effect of testosterone on thrombus formation is in accordance with previous data showing that testosterone pretreatment enhances platelet aggregability in both sexes⁸, which may be mediated through the prostaglandin system⁹.

Our results provide for the first time significant experimental evidence for an association between sex and age and the development of arterial thrombosis. These data may be relevant to epidemiological studies³ showing that arterial thrombosis and related ischaemic heart disease are less common in women during their reproductive period. The observed higher mortality rate in 12-month-old female rats seems to be comparable with the increased vascular occlusion in women after the menopause¹⁰.

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Cholecystokinin both stimulates and inhibits human food intake

THE hypothesis that gastrointestinal hormone(s) released into the blood during normal digestion should inhibit further food intake is attractive. It has been reported^{1–4} that injection of cholecystokinin (CCK) inhibited eating in rats and rhesus monkeys. We now report that intravenous infusion of CCK in a dose which produces physiological effects ascribed to CCK during normal digestion stimulated,

rather than inhibited, eating in man. In contrast, rapid intravenous injection of CCK inhibited human food intake.

Healthy, non-obese subjects (six men, four women, mean age 33 yr) gave informed consent to participate. A minor modification of the method of Jordan *et al.*⁵ for studying food intake was used. After an overnight fast, the subject ate refrigerated Ensure (Ross, Columbus, Ohio; a liquid mixture of fat, carbohydrate, protein, minerals, and vitamins, containing 1.06 kcalorie ml⁻¹) for breakfast; it was suspended in a graduated cylinder at the level of the subject's head, out of sight. It was transmitted through a No. 14 French rubber tube to the subject who ate by opening a pinchcock valve and gently sucking on the tube. Subjects were instructed to eat until their appetite was satisfied, to complete their meal within 20 min, and to report any untoward sensations. The volume of meal ingested was recorded at 1-min intervals.

Immediately before eating, subjects received an intravenous injection of either 0.15 M NaCl (control) or CCK (GIH Research Unit, Karolinska Institutet, Stockholm, Sweden; 20% pure.). The CCK dose was 0.5 Ivy dog units kg⁻¹, given over 30 s. Two to six days, during which only NaCl injections were used, were allowed for familiarisation with the procedure. After this period, the next two days were used for collection of the data reported. CCK and saline were injected in random order on these two days. The study design was double-blind. Subjects were not aware that CCK was injected only on one of the last two days.

The infusion experiment differed only in that infusion of either CCK, 3 units kg⁻¹ h⁻¹ in 0.15 M NaCl, or NaCl alone, was begun 20 min before the subject started to eat, and continued for the 20-min eating period. Thus, each subject received a total of 1 unit kg⁻¹ before, and 1 unit kg⁻¹ during, eating.

After rapid injection, CCK decreased food intake ($P < 0.025$, Table 1). The mean decrease was 54 ml (57 kcalorie; 12%). Almost all (96%) of the decrease occurred in the first 8 min (Fig. 1). During infusion, CCK increased food intake ($P < 0.05$, Table 2). The mean increase was 113 ml (120 kcalorie; 22%). An increase occurred in four of the five 4-min periods (Fig. 1).

Subjects could not distinguish CCK injections or infusions from those of NaCl. No side-effects occurred during the eating period. Within 30–60 min after the CCK infusion was stopped, three subjects had 1–3 small loose stools (Table 2).

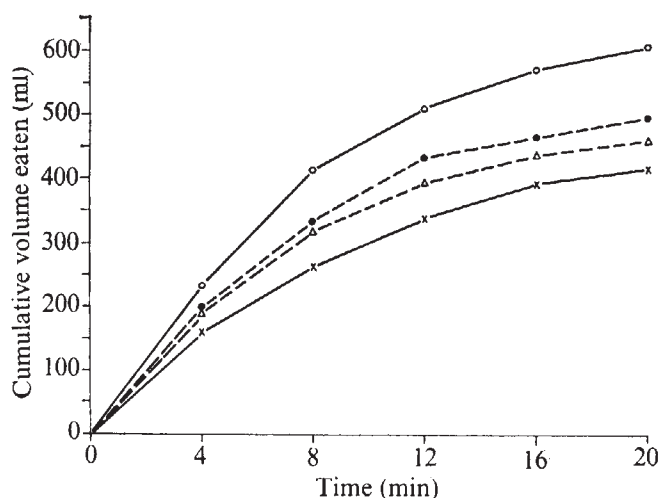


Fig. 1 Cumulative mean volume of Ensure (1.06 kcalorie ml⁻¹) eaten during CCK (○, 3 units kg⁻¹ h⁻¹) infusion (6 subjects) or after rapid CCK (×, 0.5 units kg⁻¹) injection (10 subjects). Control (0.15 M NaCl) data from the same subjects: ●, infusion control; △, injection control. Cumulative volume eaten at 20 min was significantly greater during CCK infusion ($P < 0.05$), and significantly less after CCK injection ($P < 0.025$), than with the corresponding control treatments.

Table 1 Volume (ml) eaten after injection of NaCl control or 0.5 Ivy dog unit kg⁻¹ CCK

Subject	NaCl (ml)	CCK (ml)	Difference (ml)
1	770	660	-110
2	660	560	-100
3	580	540	-40
4	520	570	+50
5	460	360	-100
6	440	440	0
7	410	410	0
8	360	230	-130
9	290	240	-50
10	180	120	-60
Mean	467	413	-54/ml

$P < 0.025$ (paired *t* test)

Diarrhoea did not occur on the control days. Side-effects did not occur in the 24 h after CCK injection. All subjects were satiated within 20 min of beginning each meal. One (injection) subject stated that the meal was not enjoyable, but she had the second largest mean intake.

The amount of inhibition in the injection study was considerably less than in the animal studies¹⁻⁴. The most obvious explanation is that our CCK dose was substantially smaller than previous doses¹⁻⁴. For example, in rhesus monkeys 5 units kg⁻¹ produced a 26% decrease in food intake, whereas 20 units kg⁻¹ produced a 70% decrease. We attempted to test 1 unit kg⁻¹, but side-effects (nausea, abdominal cramping) occurred. Although mild and transient, these effects were sufficient to destroy the double-blind design, and thus hopelessly confounded interpretation of the effects of that dose.

The infusion CCK dose was chosen to quantitatively reproduce effects on pancreatic secretion and gall bladder contraction ascribed to CCK during normal digestion⁶. Thus, it might be considered a 'physiological' dose⁷.

The occurrence of loose stools shortly after stopping the infusion suggests, however, that physiological blood concentrations were exceeded (perhaps in conjunction with endogenously released CCK), resulting in an increased rate of transit of luminal contents through the small bowel⁸. CCK blood concentrations were not determined, since no reliable method for their quantitative assay was available.

The CCK preparation used was only 20% pure, so the

Table 2 Volume (ml) eaten during infusion of NaCl control or 3 Ivy dog units kg⁻¹ h⁻¹ CCK

Subject	NaCl (ml)	CCK (ml)	Difference (ml)
1*	680	920	240
2*	660	900	240
3	530	540	10
4*	460	480	20
5	410	530	120
6	230	280	50
Mean	495	608	113

$P < 0.05$ (paired *t* test)

*1–3 loose stools after CCK.

possibility that either or both of the effects on food intake were due to impurities cannot be ruled out. In rats and monkeys, however, inhibition of food intake by CCK (from the same source as our CCK) was reproduced by the synthetic octapeptide of CCK, indicating that CCK, rather than impurities, produced the inhibition in the animal studies^{1,4}.

In summary, either stimulation or inhibition of food intake has been produced by CCK in man, depending on the dose and duration of administration. Further experiments are necessary to clarify the mechanism(s) of these effects, and their relationship to effects produced by endogenously released CCK during normal digestion.

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Methanol poisoning in the folate-deficient rat

THE recent attention given to methanol as a fuel has reawakened interest in its biological liability. Its toxicity is well known¹. Methanol poisoning is characterised in man by a latent period of 12–24 h followed by metabolic acidosis, ocular toxicity and death. This syndrome has been reported by Gilger and Potts² to occur in the rhesus monkey, but Cooper and Felig³ were unable to repeat this work. Recent studies have tended to confirm the results of Gilger and Potts. McMartin *et al.*⁴ and Clay *et al.*⁵ have shown the importance of formate generation and accumulation in the development of metabolic acidosis in the monkey, and our laboratory has been able to show ocular toxicity in the rhesus monkey⁶. On the other hand, formate does not accumulate in the rat⁴ and methanol poisoning as described for man does not occur in the rat or other small laboratory animals^{1,2}.

Palese and Tephly⁷ have shown that formate oxidation to CO₂ *in vivo* is dependent on a folate one-carbon pool pathway. Although Herken *et al.*⁸ have reported formate accumulation in blood and acidosis in the dog after methanol and amethopterin treatment, the blood pH changes were minimal ($-\Delta pH$ of 0.09 at most).

We report here the development of a profound acidosis in the rat coincident with the accumulation of high levels of blood formate after methanol treatment of folate-deficient rats. Male Sprague-Dawley rats (180–210 g) were divided into two groups fed either a folate-deficient diet or an appropriate control diet (Bio-Serve, Frenchtown, New Jersey). Liver folate levels were determined by a radioassay adapted from the method of Chanarin *et al.*⁹ with the use of kits obtained from Diagnostic Biochemistry, San Diego, California. Formate measurements were made on tail vein samples by the method of Makar *et al.*¹⁰ and ¹⁴CO₂ measurements were made as described by Palese and Tephly⁷. Blood pH was determined on samples obtained by cardiac

Table 1 Effect of folate deficiency on ¹⁴C-methanol oxidation in the rat *in vivo*

Time after methanol administration (h)	¹⁴ C-methanol oxidation (% dose)	
	Control	Folate deficiency
4	2.62 ± 0.18	1.51 ± 0.15
8	4.28 ± 0.24	2.43 ± 0.21
12	5.58 ± 0.33	3.15 ± 0.22
20	8.37 ± 0.40	4.33 ± 0.28
27	9.83 ± 0.51	5.07 ± 0.33

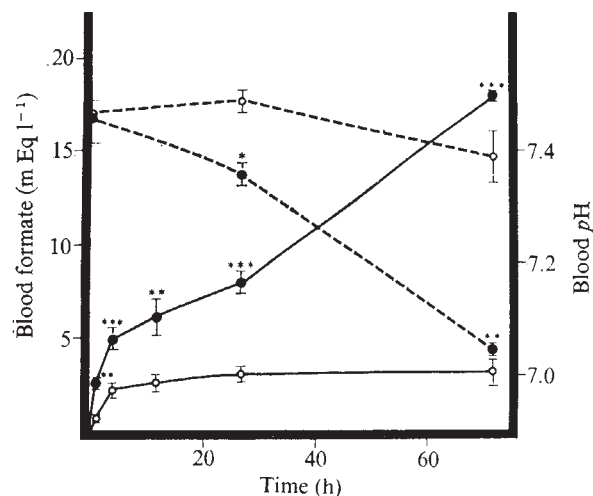
Rats received ¹⁴C-methanol (4 g kg⁻¹) intraperitoneally. Each value represents the mean of four observations ± s.e.

puncture using a blood gas analyser (Instrumentation Laboratories, Model No. 713).

Table 1 shows the rate of ¹⁴C-methanol oxidation to ¹⁴CO₂ in control and folate-deficient rats. The amount of methanol recovered as ¹⁴CO₂ was decreased to about 50% in rats fed the folate-deficient diet. Levels of liver folate were 10.26 ± 0.63 (s.e.) and 2.33 ± 0.28 (s.e.) µg per g tissue in control and folate-deficient rats, respectively. These data correlate well with the results of Palese and Tephly⁷, where liver folate levels and formate oxidation to CO₂ were reduced to a similar extent in folate-deficient rats. Figure 1 shows results for accumulation of formic acid in the blood and blood pH in animals fed on control and folate-deficient diets and which had received methanol (4 g kg⁻¹). A marked formic acidemia and acidosis ($-\Delta pH$ of 0.42, maximally) was observed in the group fed a folate-deficient diet. These results represent the first definitive demonstration of acidosis after methanol treatment in the rat, and the magnitude of changes resemble those already reported⁴ for the rhesus and pigtail monkey.

These results indicate the importance of the folate-dependent one-carbon pool in formate metabolism and its probable role in methanol poisoning. The variability in sensitivity to methanol which has been suggested for man¹¹ may be explained by the relative state of folate deficiency and functional activity of the one-carbon pool pathway. Furthermore, the species differences observed between primates and the rat may be related to this nutritional component and its biological disposition. Finally, in certain

Fig. 1 Blood formate levels (—) and pH values (---) of control (○) and folate-deficient rats (●). At zero time, methanol was administered at a dose of 4 g kg⁻¹ intraperitoneally. Each point represents the mean value obtained from 2–8 rats. Vertical bars represent ± s.e.; *, ** and *** indicate statistically significant differences from corresponding control values at *P* levels of <0.05, <0.01, and <0.001, respectively.



circumstances the folate-deficient rat may be a useful model for certain aspects of study on methanol poisoning.

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Frequency dependence of cyclic AMP in mammalian myocardium

ALTHOUGH there is now good evidence that cyclic AMP is a mediator of positive inotropic effects of catecholamines in the heart, the details of the metabolism of this nucleotide in the intact myocardial cells are still unknown. It has been shown that the intracellular concentration of cyclic AMP oscillates during each contraction cycle of the frog heart^{1,2}. But little information is available for the influence of frequency of stimulation, which regulates myocardial contractility in physiological conditions³, on the intracellular level of cyclic AMP. We therefore investigated the relationship between cyclic AMP level and the strength of contraction of the ventricular myocardium during the induction of different contractile states by changing frequency of stimulation. In order to analyse the influence of frequency of stimulation on cyclic AMP level, the effects of changes in concentrations of extracellular calcium ions or of D600, which is thought to inhibit the influx of calcium ions through the myocardial cell membrane⁴, were also investigated. It was found that the intracellular level of cyclic AMP and the contractile force of isolated rabbit papillary muscles had a reciprocal relationship during induction of different contractile states by changing frequencies of stimulation. The influence of frequency was simulated by reduction of the extracellular calcium ions or by D600.

Experiments were carried out with papillary muscles isolated from the right ventricle of rabbits of both sexes (1.3-1.9 kg). Experimental methods were the same as previously reported⁵. The papillary muscles were mounted in a 20-ml organ bath containing Krebs-Henseleit solution (NaCl, 118 mM; KCl, 4.7 mM; CaCl₂, 2.55 mM; MgSO₄, 1.18 mM; KH₂PO₄, 1.18 mM; NaHCO₃, 24.9 mM; glucose, 11.1 mM) equilibrated with 95% O₂ and 5% CO₂ at a temperature of 37 °C. The final tension of the muscles was recorded isometrically through a force displacement transducer on an ink-writing oscillograph. The papillary muscle equilibrated in the Krebs-Henseleit solution was frozen immediately in liquid nitrogen and weighed, and the content of cyclic AMP was determined by the protein binding method of Gilman⁶ as modified by Schwabe and Ebert⁷. The time required for freezing was 5-8 s.

Figure 1 shows the cyclic AMP level and the final tension of the papillary muscles stimulated at different frequencies. The tension of the papillary muscle stimulated at a rate of 1 Hz for 1 h was 416 ± 23 mg (mean ± s.e.; n=4). It was stable during the 60-min experimental period. When the

frequency of stimulation was reduced to 0.5, 0.22, 0.1 and 0.05 Hz or elevated to 2 and 3 Hz, the final tension decreased to an extent dependent on the frequency, according to the well known frequency-force relationship for ventricular muscle. The cyclic AMP level after an equilibration time of 1 h at 1 Hz amounted to 0.51 pmol mg⁻¹ wet weight of the tissue, which was stable for a further 60 min if the frequency was not changed. In contrast to the final tension the cyclic AMP level in papillary muscles was significantly high at 0.1 Hz ($P < 0.001$ compared with the value at 1 Hz) and became lower as the frequency was raised to 1 Hz. At frequencies higher than 2 Hz, the cyclic AMP level increased again and amounted to 0.72 pmol mg⁻¹ wet weight at a rate of 3 Hz ($P < 0.05$ compared with the value at 1 Hz). Thus, the cyclic AMP level of papillary muscle changed in a reciprocal relationship to the strength of contraction during stimulation at different frequencies.

Table 1 shows the influence of changes of the extracellular calcium ion concentration on cyclic AMP level and final tension of papillary muscles stimulated at 1 Hz. When the extracellular calcium ion concentration was reduced from the level of the Krebs-Henseleit solution (2.55 mM) to 0.25 or 0, respectively, the tension decreased and the cyclic AMP level rose. Treatment of the muscle with 10⁻⁶ M D600 for 30 min caused an effect similar to that of reducing the extracellular calcium ion concentration: a significant increase in cyclic AMP and a decrease in tension.

It has been shown that the uptake of radioactive calcium ions per beat increases proportionally with the strength of contraction when the strength of contraction is varied by changing the frequency of stimulation⁸. Our observations are therefore compatible with the view that the intracellular concentration of cyclic AMP, as well as the strength of contraction, may be regulated by intracellular free calcium ions. This view is reinforced further by the finding that the reduction of intracellular calcium ions probably

Fig. 1 Influence of frequency of stimulation on cyclic AMP level and tension of the isolated papillary muscle of rabbits. Papillary muscles were stretched by a tension of 0.5 g and driven electrically by a square wave pulse of 5 ms duration and a voltage about 20% above threshold at different frequencies through platinum electrodes by the use of a Hugo Sachs Elektronik Stimulator I. Muscles were equilibrated for 1 h at a frequency of 1 Hz before the frequency was adjusted to an experimental rate. The cyclic AMP level and the tension of the papillary muscle were determined when muscle tension reached the new steady level at each frequency. Numbers refer to number of papillary muscles at each point of the cyclic AMP level. Number at the right of the curve for the muscle tension represents the number of papillary muscles used for the determination of the entire course of the frequency-force relationship. ○, Muscle tension; ●, Cyclic AMP.

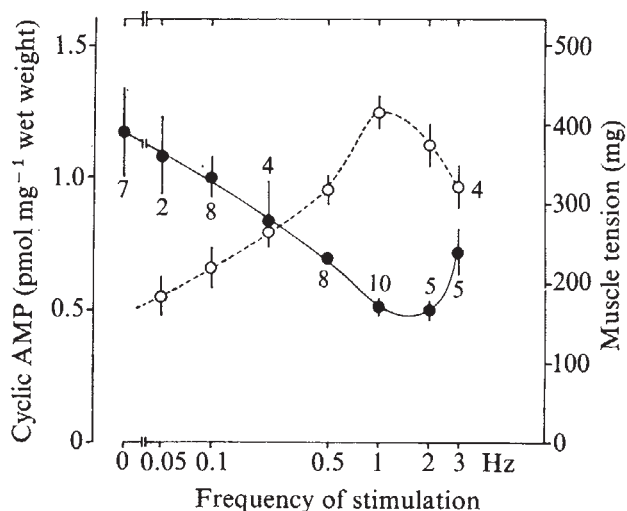


Table 1 The content of cyclic AMP and the developed tension of the rabbit papillary muscle in different conditions

	Cyclic AMP (pmol mg ⁻¹ wet wt)	Muscle tension (% of control)
Ca ²⁺ 0* mM	0.95±0.09 (7)†	6.2±0.8 (7)†
0.64 mM (×1/4)	0.85±0.19 (12)	17.9±4.1 (7)†
2.55 mM (normal)	0.51±0.04 (10)	100
D600 10 ⁻⁶ M (Ca ²⁺ 2.55 mM)	0.86±0.07 (10)†	17.9±3.5 (10)†

Influence of changes in extracellular calcium ion concentration and of D600 on cyclic AMP level and tension of isolated rabbit papillary muscle stimulated electrically at a rate of 1 Hz. The cyclic AMP level of the muscle was determined when muscle tension reached the steady level in the new experimental conditions, that is, approximately 30 min.

*No calcium was added to the medium.

†*P* < 0.001 compared with the control value in the normal calcium concentration.

induced by reducing the extracellular calcium ion concentration or by D600, caused a similar reciprocal change, that is, the elevation of the intracellular level of cyclic AMP and the reduction of the strength of contraction.

An increase in cyclic AMP in myocardial cells induced by catecholamines is fast and short-lived: the intracellular concentration of cyclic AMP returns rapidly to the control value before the peak response of contraction is reached⁹. This rapid reduction of the intracellular cyclic AMP by contrast with the gradual inotropic action of catecholamines suggests an intracellular mechanism for regulating the intracellular level of the nucleotide in the myocardium. It has recently been demonstrated that low concentrations of calcium ions stimulate phosphodiesterase from bovine hearts¹⁰ and inhibit adenylate cyclase from guinea pig hearts¹¹ in cell-free enzyme preparations. A similar regulatory role of calcium ions on the intracellular concentration of cyclic AMP is proposed also in the central nervous system^{12,13}. In the present study, however, the possibility that the increased energy requirements for tension development at higher frequencies of stimulation led to the reduction of cyclic AMP must be also considered.

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Antipsychotic drug doses and neuroleptic/dopamine receptors

ANTIPSYCHOTIC drugs, or neuroleptics, are thought to act by blocking dopamine receptors in the nervous system^{1–4}. Recent direct evidence, based on stereospecific binding assays, supports this hypothesis of antipsychotic drug action^{5–9}. As only a few antipsychotic drugs had been tested for their effects on the binding of haloperidol^{1–8}, the question remained whether all antipsychotic drugs, regardless of chemical structure, would block the stereospecific binding of haloperidol. We report here that all clinically effective antipsychotic drugs (tested so far) block the stereospecific binding of ³H-haloperidol at concentrations which correlate directly with the clinical potencies.

Binding of ³H-haloperidol was done on calf caudate homogenates, while binding of ³H-dopamine was done using rat caudate, by modifications of methods described before^{5–7}. The fresh caudate tissues were homogenised in 10 volumes of ice-cold TEAN buffer (15 mM Tris-HCl, pH 7.4, 5 mM Na₂EDTA, 1.1 mM ascorbate and 12.5 μM nialamide), using a Teflon-glass homogeniser (0.16 mm clearance; 500 r.p.m.; 15 up-down strokes). This homogenate was incubated at 37 °C for 1 h, subdivided into 200 mg wet tissue per vial, and stored frozen at –20 °C for periods of up to 3 months. Immediately before the assay, the 200-mg sample was thawed and centrifuged (15 min at 44,000g) at 4 °C. The supernatant was discarded and the pellet was resuspended in 5 ml of TEAN buffer, using a ground-glass homogeniser (five strokes by hand). The homogenate was then homogenised further using a Brinkmann Polytron (PT-10) at a setting of 7.0 (full scale = 10) for 20 s. The final protein concentration of the suspension was 1.1 mg ml⁻¹. For the binding assay, samples were added to a glass test tube (12×75 mm) as follows: 100 μl of 600 nM (+)-butaclamol or (–)-butaclamol (the final butaclamol concentration was 100 nM in the ³H-haloperidol assay, but 1 μM in the ³H-dopamine assay⁷); 100 μl of buffer containing different concentrations of antipsychotic drugs: 200 μl of ³H-haloperidol (6.4 nM, 9.66 Ci mmol⁻¹) or ³H-dopamine (3.5 nM, 8.4 Ci mmol⁻¹); and 200 μl of membrane suspension. Although the final total concentration of ³H-haloperidol was 2.14 nM, more than 50% of the drug becomes adsorbed (the drug is very membrane-soluble^{10,11}) and so the free concentration (that is the concentration of drug not bound) was actually 0.97 nM in all experiments. After incubation at room temperature (20–21 °C) for 30 min, 0.5 ml of the mixture was filtered (Whatman GF/B glass fibre filter, 2.4 cm), using a 600-mmHg vacuum, followed by washing with 5 ml of ice-cold TEAN buffer. The filters were monitored for ³H as described before^{5–7}. The stereospecific component of binding was defined as that amount of ³H-haloperidol or ³H-dopamine bound in the presence of (–)-butaclamol (inactive neuroleptic) minus that bound in the presence of (+)-butaclamol (active neuroleptic). Each experiment was done in sextuplicate, and each antipsychotic drug was tested at least three times, with most tested nine times.

At the usual *C*_{free} of 0.97 nM ³H-haloperidol, the stereospecific component of haloperidol binding was 90 fmol per mg protein of calf caudate homogenate. A Scatchard analysis (J. Tedesco and P. S., unpublished) indicated the total number of sites to be 14 pmol per g of wet tissue.

At the usual *C*_{free} of 0.97 nM ³H-haloperidol, the stereospecific blockage of haloperidol binding) are shown in Fig. 1 plotted against the average clinical doses used for controlling schizophrenia (refs for doses are in refs 12 and 13).

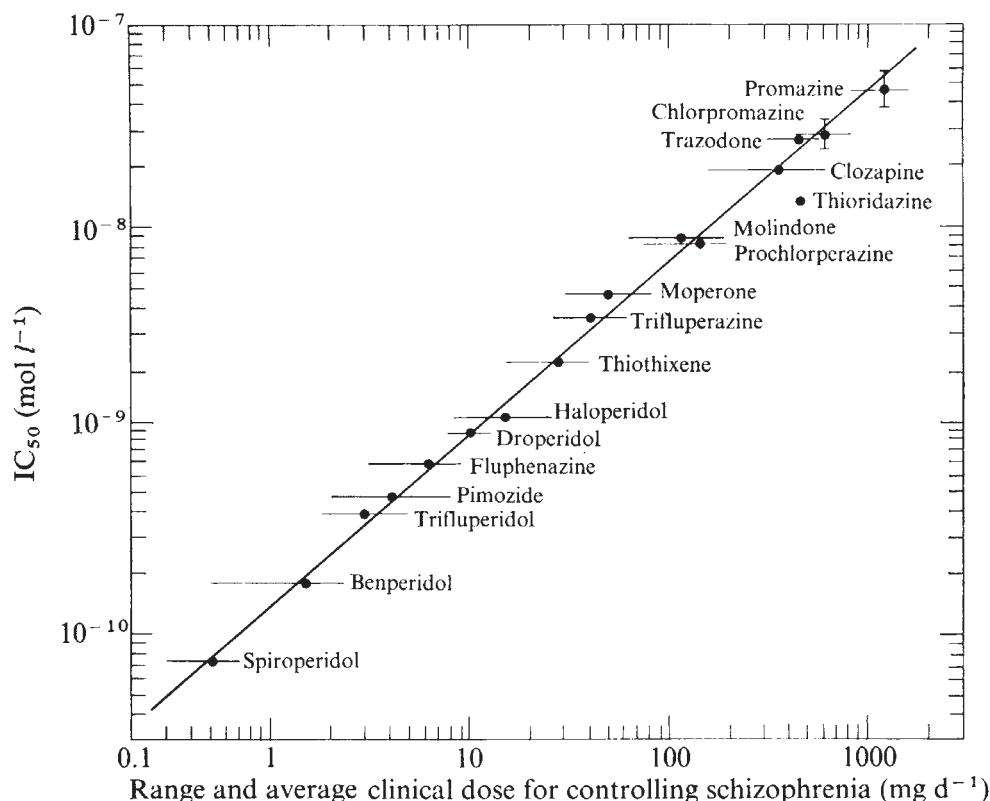


Fig. 1 The IC_{50} values (ordinate) are the concentrations of the antipsychotic drugs which reduce the stereospecific component of 3H -haloperidol binding by 50%. The abscissa indicates the average values (and ranges) of doses used for schizophrenia.

The s.e.m. for each IC_{50} is less than 10% of the IC_{50} . Further IC_{50} values for 3H -haloperidol and 3H -dopamine are

The results in Fig. 1 indicate that there is a good correlation between the antipsychotic potencies and the IC_{50} values for blocking the neuroleptic receptor. The neuroleptic receptor assay can thus be used to screen potentially useful antipsychotic drugs. The antipsychotic drugs also block the binding of 3H -dopamine, and the IC_{50} values for this effect correlate qualitatively with the clinical antipsychotic potencies, but more data are needed on this point.

The IC_{50} values may be of therapeutic value since they are almost identical to the concentrations of the antipsychotic drugs in the plasma water of the patient. For example, in the case of haloperidol being administered to a patient at doses of about 15 mg d^{-1} the plasma concentration of haloperidol is approximately 5 ng ml^{-1} (ref. 14).

Table 1 IC_{50} values for 3H -haloperidol and 3H -dopamine

	3H -haloperidol IC_{50} calf (nM)	3H -dopamine IC_{50} calf (nM) rat (nM)
Dopamine	600	9
Apomorphine	40	2
Promethazine	2,000	45
Melperone	50	
Chlorpromazine	29	2,000
Clomacran ¹⁷	28	420
Clozapine	20	200
Metiapine	15	
Molindone	9	150
Lenperone ¹⁸	20	
Haloperidol	1.2	300
Fluphenazine	0.7	30
cis-Piflutixol*	0.4	
trans-Piflutixol*	300	
p-Fluoro-spiroperidol†	0.05	

*Personal communication from I. Møller Nielsen.

†Personal communication from P. J. Janssen.

As about 10% of this is free haloperidol (unpublished data), the concentration of free haloperidol in the patient's plasma water is about 1.3 nM; this compares with the IC_{50} value of 1.2 nM. In the case of chlorpromazine, the plasma concentration is of the order of 200 ng ml^{-1} (after dose corrections^{15,16}); because 95% of the chlorpromazine is bound¹⁰, the free concentration of chlorpromazine in the plasma water of the patient would be of the order of 30 nM. This compares with an IC_{50} of 29 nM for chlorpromazine acting on the neuroleptic receptor (Fig. 1).

Finally, although the antipsychotic drugs are active in both the neuroleptic and dopamine radioreceptor assays, and although dopamine and apomorphine are active in both assays, this does not prove that the two receptors are identical, particularly since the IC_{50} values are not identical in the two assays. The agonist and antagonist ligands may also hold the receptor in a different conformation state.

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Proposed structure for brain adenylate cyclase purified using blue dextran-Sephadex chromatography

WE have recently shown¹ that blue dextran-Sephadex affinity chromatography can be used to advantage to abbreviate markedly the purification procedures for soluble enzymes whose nucleoside phosphate binding sites are constructed by a supersecondary structure called the NAD-domain. Preliminary measurements using lactate dehydrogenase indicated that affinity chromatography with blue dextran-Sephadex can also be done in the presence of various non-ionic detergents used to solubilise membrane proteins. Here we describe the use of blue dextran-Sephadex affinity chromatography for the purification of the membrane-bound hormone-responsive enzyme, adenylate cyclase, and propose a new structure for the membrane complex which does not contain a separate hormone receptor protein.

Frozen bovine brain cortex was homogenised and the insoluble material extracted with 2% Lubrol PX as described by Johnson and Sutherland². All solutions contained 5 mM NaF and 5 mM MgCl₂ and all operations were done at 4 °C to stabilise the enzymatic activity. The centrifuged Lubrol extract obtained from 1 kg of cortex was stirred overnight with 100 ml of blue dextran-Sephadex prepared by the procedure of Ryan and Vestling³. The blue dextran-Sephadex was filtered into a chromatographic column and washed with a litre of 10 mM Tris-HCl buffer, pH 7.5 containing 0.1 mM ATP. The adenylate cyclase activity was then eluted with 250 ml of the Tris buffer containing 1 mM ATP. This eluate was applied to a DEAE-cellulose chromatographic column having a bed volume of 100 ml and equilibrated with 10 mM Tris-HCl buffer, pH 8.0. The column was washed with the equilibration buffer and then eluted with a linear 0-1 M NaCl gradient contained in 500 ml of the equilibration buffer. The adenylate cyclase activity emerged as a symmetrical peak centred at about 0.9 M NaCl. Fractions containing enzymatic activity were pooled, dialysed and concentrated using an Amicon XM-50 membrane.

Results of a typical purification are given in Table 1. The specific activity of the Lubrol-extracted enzyme solution is within the range reported for detergent-extracted adenylate cyclase from cat heart⁴ and rat brain⁵ preparations. As shown in Table 1, about 50% of the adenylate cyclase activity in the Lubrol extract of bovine brain is retained by and eluted from the blue dextran-Sephadex affinity columns. This value can be increased to 65% by performing the chromatography in solvents containing 0.1 mM nor-adrenaline. The remaining 35% of the extracted enzymic activity does not bind upon reapplication to the affinity column. We assume this represents particulate bound enzyme since increasing the concentration of Lubrol increases the amount of enzymic activity retained by the affinity column. Higher concentrations of Lubrol solutions were not used, however, because of their high viscosity and resultant slow flow rates. Lubrol can be omitted from all solutions after initial solubilisation of the enzyme without adversely affecting the retention of catalytic activity.

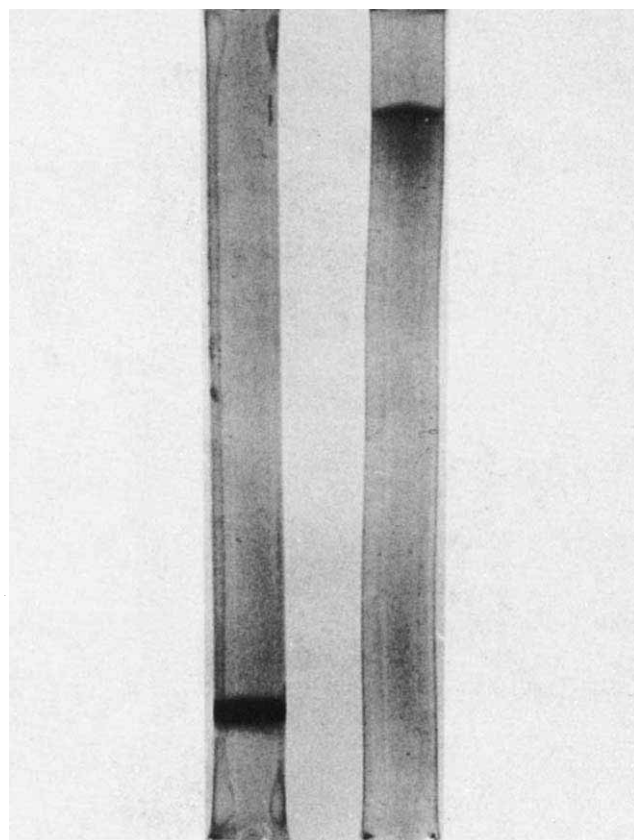


Fig. 1 Zone electrophoresis of bovine brain adenylate cyclase in polyacrylamide gels. Samples of the pooled enzymically active proteins obtained from Sephadex G-200 exclusion chromatography were applied to the top of each gel as shown. The right gel was maintained at pH 8.9 while the left gel contained 1% sodium lauryl sulphate. Electrophoresis was done for 2 h using 3 mA per gel. Both gels were stained with Coomassie brilliant blue.

About 85% of the total protein and 100% of the total catalytic activity of the material obtained by DEAE-cellulose chromatography was eluted from a calibrated Sephadex G-200 exclusion chromatographic column as a single symmetrical peak at a position corresponding to a molecular weight of $1.14 \pm 0.09 \times 10^5$ for a cytoplasmic globular protein. As shown in Fig. 1, the pooled fractions from exclusion chromatography containing enzymic activity migrate as a single component after polyacrylamide gel electrophoresis both at pH 8.9 using the procedure of Davis⁶ and in 1% sodium dodecylsulphate using the procedure of Weber and Osborn⁶. The mobility of the single

Table 1 Purification of adenylate cyclase from bovine brain cortex

Step	Volume (ml)	Total protein (mg)	Specific activity (nmol min ⁻¹ mg ⁻¹)	Yield (%)
Lubrol extract	4,500	4,210	0.097	100
Blue dextran-Sephadex chromatography	30	14	14.5	50
DEAE cellulose chromatography	35	3.5	23.6	20

Aliquots of enzyme solutions were incubated for 15 min at 37 °C in 1 mM ATP (α -³²P, about 5m Ci mmol⁻¹), 10 mM theophylline, 5 mM NaF, 5 mM MgSO₄ and 40 mM Tris-HCl buffer, pH 7.5. The catalysed reaction was terminated by rapidly heating to 100 °C. The product of the catalysed reaction, cyclic AMP, was purified by neutral alumina chromatography as described by Ramachandran⁹ and the concentration of cyclic AMP measured by liquid scintillation spectrometry. No corrections have been made in the adenylate cyclase activity measurements for the presence of theophylline-insensitive phosphodiesterases. Protein concentration was measured by the procedure of Lowry using 0.1% sodium lauryl sulphate to solubilise any Lubrol PX present¹⁰. Bovine serum albumin was used as the protein standard.

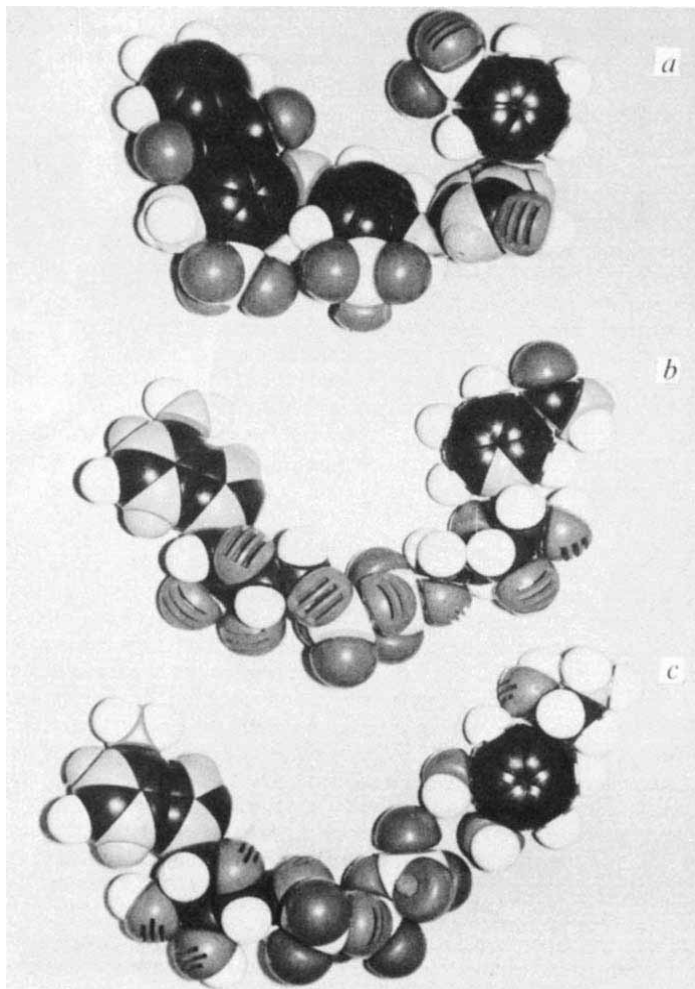


Fig. 2 Comparison of Corey-Pauling-Koltun (CPK) models of Cibacron blue F3GA (a), NAD (b) and ATP and noradrenaline (c).

component in detergent corresponds to a polypeptide molecular weight of $1.59 \pm 0.18 \times 10^4$. The catalytic activity of both the purified enzyme and the enzyme in the initial Lubrol extract is maximally stimulated twofold in the presence of 5 mM NaF and fourfold in the presence of 100 μ M of the catecholamines noradrenaline or dopamine. By contrast, the catalytic activity is not stimulated by the presence of up to 1 mM of adrenalin, serotonin, γ -amino butyrate or acetylcholine at any stage of the enzyme purification. This selective response of solubilised adenylate cyclase to hormones and neurotransmitters parallels the response of the rat brain enzyme in synaptosomal fractions⁷. The presence of both 5 mM NaF and 5 mM MgSO_4 is required to stabilise the catalytic activity of the bovine brain adenylate cyclase throughout the purification procedure. The purified enzyme is stable for at least 8 weeks when stored in 5 mM NaF at pH 7.5 and 4 °C. Since the presence of theophylline does not increase the catalytic rate of the purified enzyme, it must be free of phosphodiesterase activity.

The properties of purified adenylate cyclase suggest that the enzyme in aqueous solution contains eight identical polypeptide chains each having a molecular weight of 16,000. Since no larger catalytically active forms of the enzyme are detected, it is likely that the polypeptide chains form globular subunits arranged in a cubic quaternary structure, two subunits wide in each dimension. Assuming the protein has a v of 0.75 ml g⁻¹, the cubic quaternary structure would measure 68 Å in each dimension, a distance sufficient to span the plasma membrane.

Since adenylate cyclase binds specifically to the blue

dextran-Sepharose affinity columns, we propose that each subunit contains an NAD-domain which forms the binding site for the substrate ATP. Indeed, since formation of a typical NAD-domain requires about 150 amino acid residues⁸, the tertiary structure of each subunit must entirely consist of an NAD-domain. Comparison of CPK models for the blue chromophore of blue dextran, Cibacron F3GA, of NAD as it binds to the NAD-domain of dehydrogenases and of ATP and noradrenaline, shown in Fig. 2, indicates that the dinucleotide fold of adenylate cyclase could easily bind both the substrate ATP and the hormone in adjacent positions. As shown in Fig. 2, the ATP is oriented as the ADP portion of NAD whereas noradrenaline is oriented as the nicotinamide portion of NAD maintaining similar positions for the side chain amide of nicotinamide and the ethanolamine side chain of noradrenaline. This orientation of noradrenaline and ATP would facilitate hydrogen bonding between the two phenolic hydroxyl groups of noradrenaline and the terminal phosphate of ATP which could enhance the rate of formation of cyclic AMP in solution. Alternatively, occupancy of nicotinamide portion of the NAD-domain could promote a conformational change which would enhance the rate of catalysis.

The low concentration of ATP in the brain extracellular fluid and the absence of catecholamines in the extracellular fluid, however, precludes ligation of both ATP and catecholamine on the same NAD-domain when the enzyme is located within the plasma membrane. Accordingly, we propose that ligation of noradrenaline or dopamine to the NAD-domain of one or more of the subunits of the plasma membrane enzyme exposed to the extracellular fluid produces a conformational change which accelerates the rate of catalytic conversion of ATP to cyclic AMP at the NAD-domains of the subunits facing the intracellular fluid. Thus, a membrane bound oligomeric protein containing identical subunits could function as an allosteric enzyme without requiring a separate hormone receptor protein. Experimentation is currently being conducted to test these proposals.

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Synaptic transmission and effects of temperature at the squid giant synapse

THERE have been several reports on the effects of temperature on transmitter release at synaptic junctions¹⁻⁶. Although all reports agree that synaptic transmission is affected by temperature, the relationships are often complex and may differ from one preparation to another. The preparations studied, however, have the disadvantage that the relationship of presynaptic events (such as spike amplitude) to postsynaptic response cannot be investigated. It is possible to study this relationship at the squid giant synapse where intracellular recording can be obtained

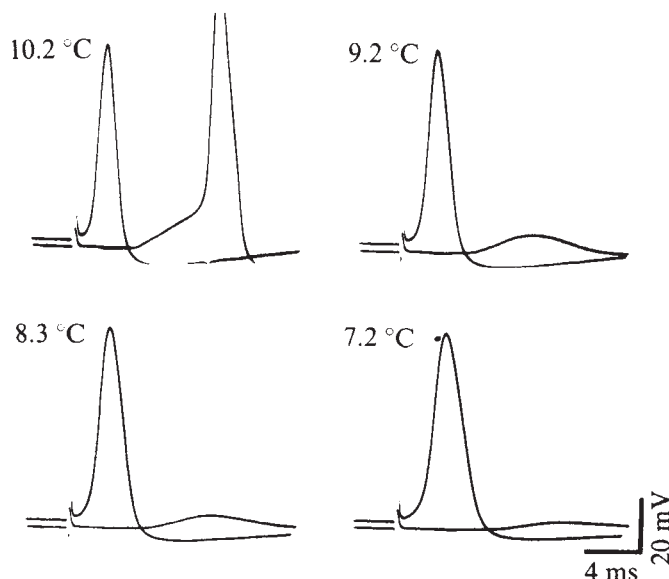


Fig. 1 Records of potentials in presynaptic terminal (upper trace) and postsynaptic axon (lower trace) of the squid giant synapse showing the effects of temperature ($^{\circ}\text{C}$) on the presynaptic action potential and the e.p.s.p. The e.p.s.p. activates a postsynaptic action potential at 10.2°C .

from both the presynaptic terminal and the postsynaptic axon. The excitatory postsynaptic potential (e.p.s.p.) at the squid synapse usually generates an action potential in the postsynaptic axon. To study the e.p.s.p., and hence transmitter release, without the postsynaptic action potential, tetrodotoxin has often been used⁷⁻⁹; this has not permitted study of the characteristics of the presynaptic spike in relation to the e.p.s.p. In a recent investigation on transmission at the squid giant synapse, we found that lowering the temperature of the preparation abolished activation of the postsynaptic action potential by the e.p.s.p., while the presynaptic spike was still generated. This observation enabled us to investigate the relationship between the presynaptic spike and the e.p.s.p. in various experimental conditions¹⁰ and we report here the effects of changing temperature.

The stellate ganglion was dissected from the squid, *Loligo pealii*, placed in a small Lucite chamber and perfused continuously with oxygenated seawater¹⁰. The temperature of the bath was controlled by a Cambion thermoelectric device and monitored continuously by a thermometer (Tri-R Instruments). In this way, the bath could be maintained at a constant temperature ($\pm 0.1^{\circ}\text{C}$) or the temperature could be changed rapidly. Micropipettes filled with 3M KCl were inserted in the mid-portion of the presynaptic terminal and in the postsynaptic axon near the synapse. Conventional electrophysiological recording techniques were used¹⁰.

At room temperature ($18-20^{\circ}\text{C}$), the e.p.s.p. in the squid axon almost always generates a postsynaptic action potential. In all of the 43 synapses investigated, when the temperature was lowered sufficiently, initiation of postsynaptic spike generation by the e.p.s.p. did not occur. The mean temperature at which the e.p.s.p. failed to activate a spike was $8.9^{\circ}\text{C} \pm 1.5$ (s.d.m.) and the range was from 5.6 to 12°C . One factor which may have a role in preventing the e.p.s.p. initiation of the postsynaptic action potential is an increase in the threshold of the action potential by the lowered temperature. Another factor which is probably more important, however, is a reduction in the e.p.s.p. amplitude produced by cooling.

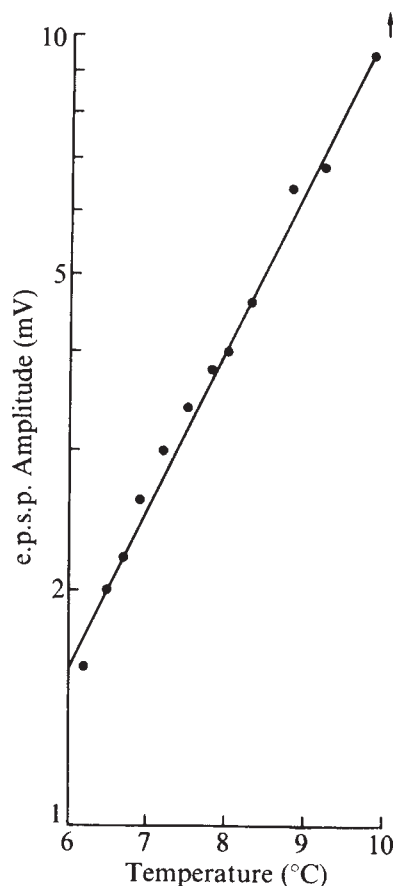
Figure 1 shows the effect of lowering bath temperature on the e.p.s.p. and the presynaptic spike. At 10.2°C (and

higher temperatures) the e.p.s.p. initiated spike generation, but as the temperature was lowered, the postsynaptic spike was not activated and the e.p.s.p. amplitude decreased progressively. This was accompanied by an increase in the width of the presynaptic spike, due to a decrease in the rate of rise and fall of the action potential. This effect of temperature has been described previously for the action potential of the squid giant axon¹¹. Finally, an increase in synaptic delay can also be seen, as reported previously¹².

Figure 2 shows a graphic representation of the change in e.p.s.p. amplitude as a function of temperature. There is a linear relationship between the log of the e.p.s.p. amplitude and temperature. The slope of the curve indicates a high degree of temperature sensitivity for transmitter release. From an Arrhenius plot of the log of the e.p.s.p. amplitude against the reciprocal of temperature (K), an apparent activation energy of 74 kcalorie per degree can be calculated. This compares with an activation energy of 25 kcalorie per degree reported³ for transmitter release at the rat phrenic nerve-diaphragm synapse.

The relationships between e.p.s.p. amplitude, presynaptic spike height and temperature are plotted for comparison on a linear scale in Fig. 3. Figure 3a shows the relationship between e.p.s.p. amplitude and temperature; the slope of the curve is steepest in the temperature range between 9 and 10°C . The relationship between presynaptic spike height and temperature is illustrated in Fig. 3b. In contrast to the curve relating e.p.s.p. amplitude to temperature, the presynaptic spike height curve has the greatest change in the range 6 to 8°C . To assess whether the change in transmitter release at different temperatures is due to the effects of temperature on presynaptic spike height, the direct relationship between presynaptic spike height and e.p.s.p. amplitude at different temperatures is plotted in Fig. 3c.

Fig. 2 Graph of relationship between log of e.p.s.p. amplitude (mV) and temperature ($^{\circ}\text{C}$) at the squid giant synapse. From the same experiment illustrated in Fig. 1.



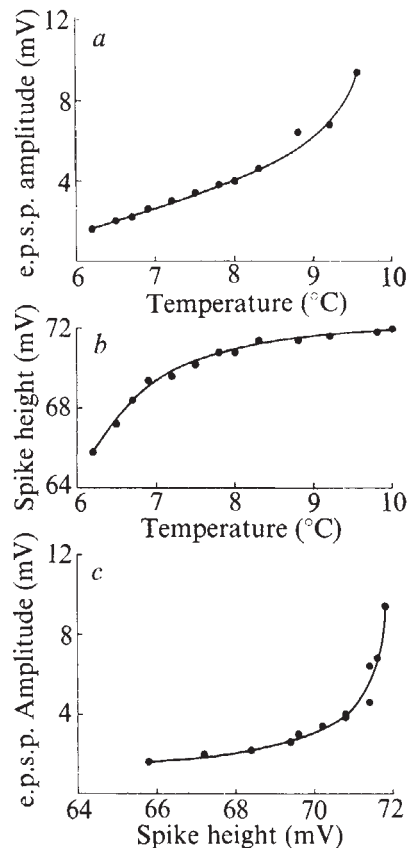


Fig. 3 Relationship between e.p.s.p. amplitude, presynaptic spike height and temperature (°C) plotted on linear coordinates. a, e.p.s.p. amplitude (mV) plotted as a function of temperature. b, Presynaptic spike height (mV) plotted as a function of temperature. c, Graphic relationship between e.p.s.p. amplitude (mV) and presynaptic spike height (mV) at different temperatures. From the same experiment illustrated in Figs 1 and 2.

This relationship does not agree with the relationship between presynaptic spike height and e.p.s.p. amplitude reported in previous studies using electrical polarisation of the presynaptic terminal^{9,13-15}. This discrepancy suggests that changes in the presynaptic spike height may not be a major factor in the effect of temperature on transmitter release. The effect of temperature on transmitter release at the squid synapse is therefore likely to involve other factors such as temperature sensitivity of the release mechanism.

In summary, lowering the temperature of the squid giant synapse prevents e.p.s.p. initiation of the postsynaptic action potential and permits investigation of the relationship between the e.p.s.p. and the presynaptic action potential. An investigation of the effects of temperature on transmitter release reveals that transmitter release is temperature sensitive; from an Arrhenius plot an apparent activation energy of 74 kcalorie per degree, was calculated. Finally, a change in presynaptic spike height does not seem to be the major factor involved in the sensitivity of transmitter release to temperature.

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Stimulation of active Na transport by phospholipase C

We have found that an enzyme, phospholipase C, which has a very specific action in cleaving phosphatidylcholine (lecithin) at its ester linkage (between the α carbon of glycerol and the phosphoryl group of phosphorylcholine) stimulates active Na transport across frog skin *in vitro*. This action may provide both insight into the sort of processes involved in transepithelial Na transport and a model for the action of proteins induced by aldosterone^{1,2}. This hormone stimulates Na transport across epithelial membranes which, apart from the kidney tubules, include *in vitro* frog skin, and toad urinary bladder and colon³⁻⁷.

When phospholipase C (Sigma, batch no. 84C-6871, Type I from *Cl. welchii*) was placed in the solution bathing the corium side of frog (*Rana pipiens*) skin, it produced a sustained increase in the short-circuit current (SCC), normally reflecting active Na transport, and transmembrane potential difference (Fig. 1). The peak effect was seen in about 30 min and the increase was maintained for several hours. When the enzyme was washed from the tissue, the SCC returned to the initial levels after about 1 h, suggesting that the membrane can reconstitute its original character. Phospholipase C placed on the epidermal side of the frog skin also produced an increase in the SCC, but its effect was much less. The activity of the phospholipase preparation was destroyed by immersing it in a boiling water bath for 10 min, which is consistent with its action being due to the enzyme. The SCC in the presence of phospholipase C was abolished by amiloride (10^{-4} M), which is a specific antagonist of active Na transport across such epithelia^{8,9}.

Cuthbert and Painter¹⁰ have shown that phospholipase C produces an "initial stimulation" of SCC in frog skin, but this increase was followed by a prominent decline. To maintain the activity of the enzyme, they bathed the skin in non-aerated Ringer solution, a precaution which we found unnecessary, which probably accounts for the differences in the results. We have found that in the toad urinary bladder phospholipase C (on the serosal side) causes a small initial increase in SCC, which then declines. On the vitreous side of the toad crystalline lens it produced a sustained increase in the translenticular SCC.

Active Na transport across amphibian epithelia can be stimulated by several hormones and drugs, including anti-diuretic hormone (ADH), aldosterone, and amphotericin B. The latter increases Na transport across the toad urinary bladder and it has been suggested to act analogously, and so serve as a model, to aldosterone¹¹. Amphotericin B reacts with sterols in the walls of cells to create pores, or channels, through which Na⁺ can pass. Its effect, however, cannot be inhibited by amiloride⁹, and there is an increase in both the influx and efflux of Na⁺ across the membrane¹². In contrast aldosterone and ADH increase the influx but have little or no effect on the efflux.

We measured the effects of phospholipase C on the unidirectional fluxes of ²²Na, and found that while the influx increased there was no change in the efflux (Table

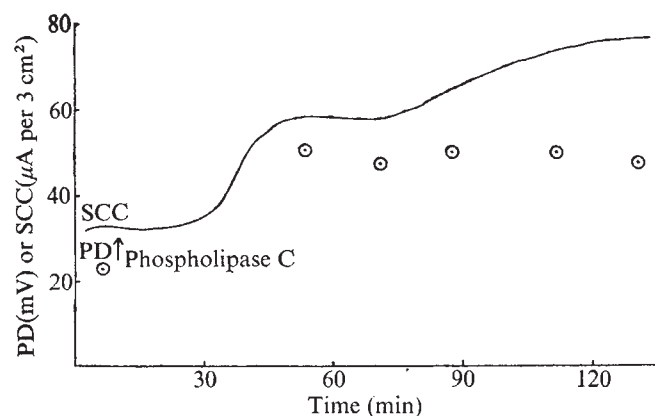


Fig. 1 Effects of phospholipase C ($50 \mu\text{g ml}^{-1}$, 8.5 U mg^{-1} on the corium side) on the SCC ($\mu\text{A cm}^{-2}$) and the potential difference (p.d.) (mV) across frog skin *in vitro*.

1). The SCC remained equivalent to the net Na transport. Thus phospholipase C acts specifically to increase Na influx, which is the direction of the active Na transport. Its effect is to create 'one-way' (amiloride-inhibitable) rather than 'two-way', nonspecific, channels in the membrane. The precise site of its action, however, is unknown, and it could be either increasing the access of Na to the pump mechanism or acting directly on the latter.

To explore further the relationships and analogies of the action of phospholipase C with that of natural hormones its effects were measured in the presence of ADH and aldosterone. The actions of vasotocin (the natural ADH in amphibians) and aldosterone appeared to be additive with those of phospholipase C (Table 2). This observation confirms the physiological integrity of the normal active Na transport mechanism in the presence of the enzyme. It also suggests that the precise sites involved in the responses to the hormones and the exogenous enzyme may differ.

These results are principally interesting with respect to the concept of possible mechanisms that may control active Na transport and account for its stimulation, such as by hormones like aldosterone. They show that an enzyme with a highly specific action can, reversibly, increase active Na transport by promoting influx without changing the efflux. Phosphatidylcholine could be the site of such an action and splitting of its ester linkage may be involved. This cleavage of the molecule would create free polar groups with which Na could interact, and this is in agreement with a proposed mechanism for transepithelial Na transfer in frog skin¹³. As

Table 2 Interactions in the responses of frog skin to phospholipase C, vasotocin and aldosterone

	SCC ($\mu\text{A cm}^{-2}$)		
	Initial	Final	Increase
(1) Vasotocin			
Control, vasotocin alone	23	35	12 ± 1.5
Vasotocin in presence of phospholipase C	34	48	14 ± 3.1
(2) Aldosterone			
Control, phospholipase C alone	17	31	14 ± 2.5
Phospholipase C in presence of aldosterone	40	57	17 ± 0.8

The frog skins were prepared as described in Table 1. In experiment (1) phospholipase C ($50 \mu\text{g ml}^{-1}$) was added, and after a stable response was seen, vasotocin ($10 \text{ mU pressor per ml}$ in a maximal dose) was placed on the corium side and the maximum increase in SCC was recorded. The effect of vasotocin on a paired control preparation was measured at the same time. In experiment (2) paired skins were incubated for about 18 h, one in the presence of aldosterone (10^{-7} M , both sides) at which time a peak stable response (= initial) to the hormone was seen. The enzyme was then added to this preparation as well as to the control preparation and the succeeding maximal stable values recorded.

it is unlikely that the phospholipase C enters the cells, it seems to be acting on their external surface. Such an effect could occur *in vivo* if an enzyme were secreted from specialised cells, such as the mitochondria-rich cells which have been proposed as a locus of hormonal action in the frog skin¹⁴ and toad urinary bladder¹⁵. Such a secretory product could function as a lateral transmitter and stimulate Na transport in neighbouring cells. The results also suggest that proteins, such as those induced by aldosterone, that may stimulate active Na transport, could be similar to phospholipase C.

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Table 1 Effects of phospholipase C on unidirectional fluxes of ^{22}Na and the potential difference and SCC across frog skin *in vitro*

	Initial	Phospholipase C present	Mean difference
Na influx ($\mu\text{Eq cm}^{-2} \text{ h}^{-1}$)	1.02	1.45	$0.43 \pm 0.08^*$
PD (mV)	24	39	$15 \pm 2.9^*$
SCC ($\mu\text{A cm}^{-2}$)	22	36	$14 \pm 2.1^*$
Na efflux	0.29	0.26	0.03 ± 0.03
PD	18	34	$16 \pm 2.3^*$
SCC	20	37	$17 \pm 2.4^*$

* $P < 0.01$ for mean difference.

The ventral abdominal skins (3 cm^2) were mounted in divided Ussing-type chambers and bathed on both sides, at $21\text{--}22^\circ\text{C}$, with Ringer solution containing (mM): NaCl, 111; KCl, 3.35; CaCl_2 , 2.7; NaHCO_3 , 4.0 and glucose, 5.0. The solutions on both sides were aerated and the pH was 8.3. Apart from briefly reading the potential difference (p.d.), the skin was maintained in short-circuited conditions with an automatic voltage clamp. Each flux value represents the mean of four 15-min periods. The results are expressed as the mean difference $\pm$ s.e. for six experiments per unidirectional flux.

Membrane retrieval at neurosecretory axon endings

EARLY descriptions of neurohypophysial hormone release by exocytosis¹ suggested that, after exocytotic discharge of the content of the neurosecretory granules (NSG), the membrane of the granule was recaptured in the form of electron-lucent

microvesicles of diameter ~ 50 nm. Such microvesicles were also shown to take up the extracellular marker horseradish peroxidase². Larger vacuoles of a diameter similar to that of NSG have also been shown to take up this marker in stimulated neurohypophyses^{3,4}. Furthermore, reuptake of membrane into organelles of approximately the size of NSG has also been predicted on the basis of studies of NSG-membrane labelling⁵ and radioactive extracellular tracer uptake⁴. We have studied membrane retrieval after stimulation of the rat neurohypophysis by stereology^{6,7} in an attempt to assess the relative contribution of vacuoles and microvesicles to the membrane reuptake process. Depletion of neurosecretory granules was associated with membrane retrieval by vacuoles of similar size. There was no detectable change in the microvesicle population.

Albino rats of 300–350 g body weight were decapitated, and the neural lobes were removed and incubated for 20 min in modified Locke's solution (150 mM NaCl, 2.2 mM CaCl_2 , 1 mM MgCl_2 , 5.6 mM KHCO_3 , 10 mM glucose, pH 7.3) which was gassed continuously with 5% CO_2 in O_2 . Glands were next incubated for 5 min in this solution where choline had been substituted for sodium. After this stabilisation period, the lobes were incubated for 15 min in either a high-potassium (56 mM K^+ ; 0 mM Na^+) Locke's solution to stimulate release of oxytocin and vasopressin, or in control (5.6 mM K^+ , 0 mM Na^+) solution. The hormone released was assessed by measuring the oxytocin content of the medium, determined by a rat bioassay⁸.

Point counting volumetry was performed on a sample of 201 profiles of nerve endings from six control glands and 207 profiles of endings from six stimulated glands ($\times 43,800$). The endings were selected by reference to their position on the grid, and the micrographs assessed with the observer unaware of the source of the micrographs. For the purpose of this study a nerve ending was defined as a dilatation of a neurosecretory axon containing microvesicles in addition to NSG. Many profiles of such endings transected their contact zone with the basement membrane while others did not. Vacuoles were defined as electron-lucent vesicles more than 80 nm in diameter (Fig. 1a and b); their sectioned profiles were not always perfectly circular and, in stimulated glands (Fig. 1b), they occasionally contained a very small amount of electron-dense material. They differed from aldehyde-fixed NSG which had lost core electron density as a result of inappropriate fixation conditions⁹ by virtue of their continuous trilaminar membrane and the electron lucency of their content, which was similar to that of the interior of microvesicles.

The results (Table 1) indicate that, in the endings, after stimulation of hormone release, the proportion of the total volume occupied by NSG is significantly ($P < 0.001$) decreased while that of vacuoles is significantly increased ($P < 0.001$). The vacuoles were significantly larger ($P < 0.001$) in stimulated than in control glands (Kolmogorov-Smirnov test) but this increase in size is insufficient to account for the increased volume that they occupy. The results therefore indicate that after release has been stimulated, the NSG decrease in

Fig. 1 Electron micrographs of rat neurohypophyses ($\times 45,270$). a, Control (5.6 mM KCl); b, stimulated neurohypophysis (56 mM KCl). The arrows indicate membrane-bounded vacuoles.

*Microvesicles; NSG, neurosecretory granules.

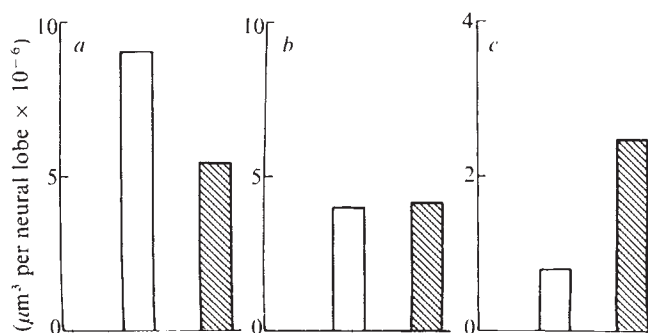
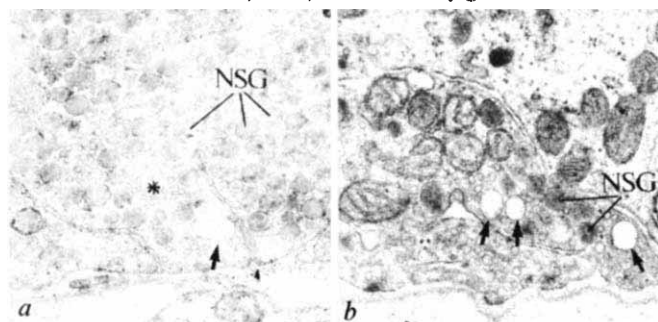


Fig. 2 Calculated content of NSG (a), microvesicles (b) and vacuoles in the endings (c) of the neural lobe. The values are calculated from the volume proportion data assuming that the endings comprise 12.07% (ref. 13) of a total embedded neural lobe volume of 0.566 mm³ (ref. 11). The Holmes correction has been applied to the neurosecretory granule data. Open columns, control experiments (5.6 mM KCl); hatched columns, stimulated preparations (56 mM KCl).

number and the vacuoles increase both in number and in size (Fig. 2); by contrast neither the microvesicle (Fig. 2) nor the mitochondrial population was altered significantly. Furthermore, it should be pointed out that after hormone release there is no increase in the surface area of the endings. Depletion of NSG was greatest in those profiles of endings where contact with the basement membrane was included (Table 1), a finding consistent with exocytosis at this contact zone. Because, after stimulation, the vacuoles were found equally in profiles which did and did not transect the contact zone, it seems either that they are rapidly dispersed through the endings or that membrane reuptake does not occur exclusively at the release site. Such a rapid dispersal together with the low basal hormone turnover in the unstimulated neural lobe¹⁰ would explain the paucity of vacuoles in endings from control glands.

The amount of hormone released from one neurohypophysis during 15 min of incubation in the high- K^+ solution was 128 mU. Since each NSG contains 6×10^{-8} mU hormone¹¹, such a release would be represented by the content of 2.1×10^9 NSG. We calculate from the stereological data that the granule depletion from the endings alone represents a loss of 1.7×10^9 NSG, and redistribution of NSG within the neurosecretory process would undoubtedly make this a minimum figure for the total granule loss. There is thus surprisingly good agreement between the data derived by stereology and bioassay.

After release there is no detectable change in the volume

Table 1 Comparison of the proportional volume occupied by neurosecretory granules, microvesicles and vacuoles in neurosecretory endings of the neural lobe in control (5.6 mM K^+) and stimulated (56 mM K^+) preparations

		Control (5.6 mM K^+)	Stimulated (56 mM K^+)
Neurosecretory granules	ALL	18.8 $\pm$ 0.9 (201)	11.9 $\pm$ 0.9 (207)*
	BM+	17.6 $\pm$ 1.1 (137)	8.7 $\pm$ 0.8 (137)*
	BM-	20.7 $\pm$ 1.8 (64)	20.3 $\pm$ 2.1 (70)
Microvesicles	ALL	7.2 $\pm$ 0.5 (201)	7.3 $\pm$ 0.6 (207)
	BM+	6.8 $\pm$ 0.6 (137)	8.2 $\pm$ 0.8 (137)
	BM-	7.8 $\pm$ 1.0 (64)	5.4 $\pm$ 0.9 (70)
Vacuoles	ALL	1.2 $\pm$ 0.4 (201)	3.6 $\pm$ 0.5 (207)*
	BM+	1.6 $\pm$ 0.6 (137)	4.2 $\pm$ 0.7 (137)*
	BM-	0.2 $\pm$ 0.1 (64)	2.2 $\pm$ 0.5 (70)*

Values are percentages of nerve ending profile area occupied by the organelle (mean $\pm$ s.e.m.). The number of endings studied is given in parentheses. ALL, data for all nerve endings; BM+, data for those nerve ending profiles transecting the contact with the basement membrane; BM-, data for those ending profiles not transecting the contact with the basement membrane.

*Difference between control and stimulated, $P < 0.001$ by Student's t test.

occupied by microvesicles, but the volume of uptake by the newly formed vacuoles in the endings would be $1.5 \times 10^6 \mu\text{m}^3$ per neural lobe. This would represent about 41% of the volume of the NSGs released and is in fairly good agreement with the reported volume uptake ($3.1 \times 10^6 \mu\text{m}^3$) determined by extracellular tracers⁴.

The results therefore indicate that a major route for the recapture of membrane after exocytosis of NSG is by vacuoles of a similar size to NSG, but do not demonstrate any significant recapture by microvesicles. They thus contrast sharply with the findings of Nagasawa *et al.*² who report that, in the rat and hamster neurohypophysis, stimulation of hormone release results in uptake of horseradish peroxidase into microvesicles; they did not, however, quantify this effect. In the rat neurohypophysis, incubation in depolarising conditions results in an uptake of horseradish peroxidase and radioactive extracellular markers into the nerve endings⁴. Because this uptake was abolished when hormone release was blocked by Ca^{2+} antagonists, this is consistent with the concept of an exocytosis-endocytosis coupling mechanism.

In conclusion, our results highlight the fact that the model of membrane retrieval exclusively by way of microvesicles at neurosecretory axon terminals^{2,12} cannot be generalised. Indeed our results show that after stimulation of hormone release there was no detectable change in the microvesicle population. Neurohypophysial vacuoles^{3,4} can be a major route for recapture of membrane. The increase in size of the vacuoles after stimulation suggests that the size of the membrane-recapture organelle is a function of the intensity of secretion. Although larger vacuoles would be less efficient at storing membrane in a given volume, it would be expected that membrane retrieval into the larger organelles would be energetically a less costly process and would have the advantage of maintaining the volume of the endings relatively constant in the acute situation.

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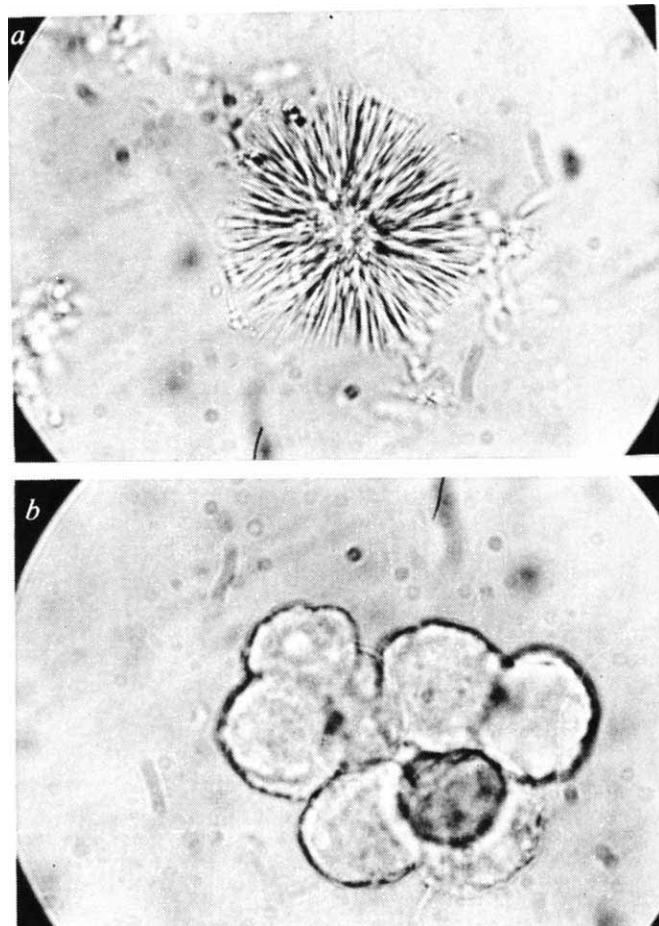


Fig. 1 Photomicrograph of crystals of DNA-dependent RNA polymerase (core enzyme) from *T. thermophilus* HB 8: *a*, needle crystals; *b*, spherulites (about $\times 400$). For crystallisation, the core enzyme (5 mg ml^{-1}) was dialysed in a small dialysis bag against 0.01 M Tris-HCl , 0.01 M MgCl_2 , 0.1 mM EDTA , 2-mercaptoethanol , containing 25% saturated (at 25°C) ammonium sulphate, $\text{pH } 8$. The dialysis bag was placed in a test tube together with 15 ml of the dialysate. An appropriate amount of the saturated ammonium sulphate solution, made by further addition of solid ammonium sulphate to the dialysate, was added without stirring the tube, so that the final concentration of ammonium sulphate after equilibrium becomes 55% or more. The test tube was covered with polyethylene film and left at 25°C . Crystallisation was completed in a few weeks.

Crystallisation of DNA-dependent RNA polymerase from *Thermus thermophilus* HB 8

BACTERIAL DNA-dependent RNA polymerase (nucleoside triphosphate-RNA nucleotidyltransferase; EC 2.7.7.6) consists of at least four different subunits (α , β , β' , and σ) and the subunit composition of the holo- and core enzymes are $\alpha\beta\beta'\sigma$ and $\alpha\beta\beta'$, respectively. These subunits play different roles and the overall reaction catalysed by this enzyme is well organised and highly specific. To understand the mechanism of transcription, a precise knowledge of the structure of this enzyme is indispensable. The structure of RNA polymerase has been studied by small-angle X-ray analysis¹

and electron microscopy^{2–6}. Data obtained using these methods, however, are not satisfactory, and even contradict each other. X-ray crystallography seems to be^{7,8} the most powerful method of analysing the three-dimensional structure of proteins. Since RNA polymerase has not yet been crystallised, however, it has been impossible to apply this technique. We have recently isolated the core enzymes and holoenzymes of RNA polymerase from the thermophilic bacterium, *Thermus thermophilus* HB 8 (ref. 9). Both enzymes are quite stable not only against heating but also against denaturation by various detergents. One of the reasons why crystallisation of RNA polymerase has not yet been achieved in spite of many publications concerning extensive purification of the enzyme from various sources, is probably the instability of the enzyme. As a first step towards X-ray crystallographic analysis of RNA polymerase, we tried to crystallise the thermophilic core enzymes and holoenzymes in the hope that their extreme stability might help the crystallisation of the enzyme. We have succeeded in crystallising the core enzyme but not the holoenzyme.

The thermophilic RNA polymerases, holo- and core enzymes, were isolated from *T. thermophilus* HB 8 as

described previously⁹. The purified holoenzymes and core enzymes were homogeneous as judged by gel electrophoresis, isoelectric focusing and ultracentrifuge studies. As described previously, however, two types of the core enzymes exist. Their subunit composition is $\alpha_2\beta\beta'$ and $\alpha_2x\beta'$, respectively, where the molecular weights of α , β , β' , and x subunits are 42,000, 140,000, 180,000 and 100,000, respectively. From the results of our reconstitution experiments, it was assumed that subunit x has the same function as that of subunit β and is derived from subunit β probably by proteolytic digestion (our unpublished results). These two core enzymes cannot be distinguished by specific activity or by template specificity.

In the following crystallisation procedure, the core enzyme fraction (3 : 1 mixture of $\alpha_2\beta\beta'$ and $\alpha_2x\beta'$) was used without further fractionation, because the two core enzymes could not be separated easily⁹. Crystals of the core enzyme grow in two forms (Fig. 1)—needle crystals (*a*) and spherulites (*b*). These two forms of crystals were extensively washed with the mother liquor and dissolved in buffer (see Fig. 2). The enzyme solutions were analysed for subunit composition by SDS polyacrylamide gel electrophoresis and tested for enzyme activity. The patterns of SDS-polyacrylamide gel electrophoresis of the two crystal forms (Fig. 2) were almost identical. The same was true for the core enzyme fraction used for crystallisation, indicating

Fig. 2 SDS-polyacrylamide gel electrophoresis of crystals and the original core enzyme of RNA polymerase from *T. thermophilus* HB 8: *a*, core enzyme; *b*, needle crystals; *c*, spherulites. After washing extensively with the mother liquor, the two forms of crystal were dissolved in 0.01 M Tris-HCl, pH 8.0, 0.01 M MgCl₂, 0.1 mM EDTA, and 0.1 mM dithiothreitol, and analysed by SDS-polyacrylamide gel electrophoresis (concentration of acrylamide 7.5%) according to Weber and Osborn¹⁰. After electrophoresis, gels were stained with Coomassie brilliant blue, and traced at A_{620} with a Joyce Loebl Chromo Scan FS 200. BPB indicate the position of the bromophenol blue marker dye.

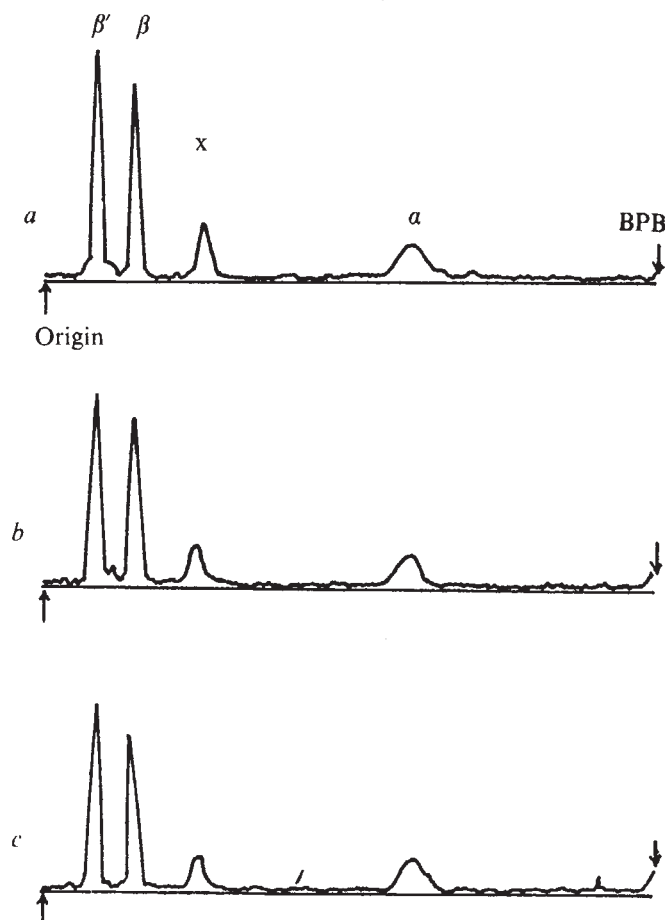


Table 1 Specific activity of the core enzyme of RNA polymerase from *T. thermophilus* HB 8, and its crystals

	Specific activity ($\times 10^{-4}$ U mg ⁻¹)
Core enzyme of RNA polymerase	2.12
Needle crystals	1.71
Spherulites	1.55

The two forms of crystal were dissolved in buffer as described in Fig. 1, and these solutions were assayed with poly d(A-T) as template. The assay mixture (0.10 ml) contained 0.05 M glycine-NaOH, pH 8.2, at 65 °C, 10 mM MgCl₂, 2 mM MnCl₂, 0.1 mM EDTA, 0.1 M KCl, 0.01 M 2-mercaptoethanol, 0.4 mM each of ATP, GTP and CTP, 0.4 mM ³H-UTP (1–2 mCi mmol⁻¹) and 4 μ g poly d(A-T) and 1 μ g enzyme. After incubation for 10 min at 65 °C the reaction mixture was chilled in ice-cold 10% TCA. The precipitates were collected on a Millipore filter, and washed with ice-cold 5% TCA. The filters were immersed in a toluene based scintillation fluid and the radioactivity was counted with a Packard Tri-Carb 2425 liquid scintillation spectrometer. One unit of RNA polymerase activity is defined as the amount of protein which catalyses the incorporation of 1 nmol UMP into RNA in 60 min at 65 °C in the conditions described above. The amount of protein was calculated from A_{280} and A_{260} according to Kalckar¹¹.

that both core enzymes crystallised in two forms. Since the amount of x in both crystal forms seems, however, to be slightly lower than that in the original fraction, the $\alpha_2\beta\beta'$ type core enzyme may crystallise more easily than the $\alpha_2x\beta'$ type core enzyme. Specific activities of crystals (Table 1) were slightly lower than that of the original core enzyme, but it can be concluded that these crystals have the core enzyme activity.

These results indicate that both are crystals of the core enzyme of DNA-dependent RNA polymerase from *T. thermophilus* HB 8, and have essentially identical compositions and activities. Protein crystals which grow as hedgehog-like aggregates of needle crystals (Fig 1a) frequently develop into spherulites (Fig. 1b).

To obtain core enzyme crystals suitable for X-ray analyses and to crystallise the holoenzyme precipitated in the above conditions, further improvement of the conditions for crystallisation is necessary.

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Nature Index and Binders

The **Index** for 1975 is now available, price £2.25. Copies of the 1974 index are still on sale, price £3.00. **Binders** for the journal are also available at £7.00 for four (a year of *Nature* fits into four binders).

Postage is included in the above prices. Orders should be sent, accompanied by remittance to Macmillan Journals Ltd, Brunel Road, Basingstoke, Hampshire, England.

reviews

Forests and their genetics

Tropical Rain Forests of the Far East. By T. C. Whitmore. With a chapter on soils by C. P. Burnham. Pp. xiii+282. (Clarendon: Oxford; Oxford University: London, December 1975.) £12.50.

THIS is a superb book. I dearly wish that it had been available before I made my first visit to a tropical rain forest. The title is accurate but unduly modest. The book indeed treats tropical rain forests of the Far East exhaustively, but in a surprisingly compact and literate form. The author's approach, however, is so broadly interpretive that much of what he says gives equal insight into other tropical forests.

In addition to chapters on geography, climate, and forest structure, there are excellent separate chapters and dispersed notes on silviculture, animals, and the interactions between plants and animals.

Much of the interpretive study of tropical forests has suffered from over-generalisation followed by nihilistic reaction. Whitmore gives refreshingly balanced discussions of topics such as storied vegetation, lateritic soils, buttressed roots, and compound leaves. He reviews recent theories of the structure and economy of tropical forests with a healthy mixture of enthusiasm and agnosticism. His extensive field experience is apparent in many practical hints about what one should look for when identifying trees or reading a landscape.

The book is abundantly illustrated with elegant photographs, each taken under technically taxing conditions and each showing its ecological point remarkably well. Cross-references among chapters are unusually apt and easy to follow. The bibliography is copious, selected with acute judgement, and up to date within a year of its publication. The free use of botanical cryptology is a deterrent to the wide 'browsership' that the book deserves; but it is not an insurmountable difficulty, since arcane terms are defined where they first occur. A glossary would have helped.

Whitmore ends most of his critical discussions with an explicit list of data that are needed to evaluate interpretations of previous observations. His book should therefore be especially valuable to graduate students and ad-

vanced undergraduates. It is both successor and complement to P. W. Richard's classic *The Tropical Rain Forest* (1952, Cambridge University Press).

A final section on human use is a succinct, rational, and eloquent plea for conservation of some remaining unexploited forests, and for applying natural history and common sense to the management of exploited areas.

Henry S. Horn

Introduction to Forest Genetics. By Jonathan W. Wright. Pp. xvi+463. (Academic: New York and London, February 1976.) \$19.50; £10.15.

STUDY or use of genetic variation in forest trees began early in the last century but systematic application of genetic principles to the domestication of trees began only 50 years ago. Through the last 30 years there has been a dramatic increase in activity with programmes for population testing and selective breeding being initiated in many countries, with many species, and for many different types of forest products.

Professor Wright has long been one of the leading practitioners and teachers of forest genetics and tree improvement in North America where, together with northern Europe, most of the early work was done and where the larger and most advanced programmes are located. One of the few criticisms of this book arises from that; the bulk of the examples and the references quoted are from the temperate regions of North America and Europe with some from Japan, New Zealand and South America; tropical species and areas in which there is now much activity and rapid progress, receive scant attention. Although this may detract from the value of the book as a worldwide review it does not affect the principle aim, which is to provide an introductory text for future professional tree breeders.

In 1962, at the request of the United Nations Food and Agriculture Organisation Professor Wright prepared *Genetics of Forest Tree Improvement* (FAO Forestry and Forest Product Studies No. 16, Rome). Until now this has remained the only textbook in English on the subject and for several years it has been unobtainable.

The present book is a revision of the 1962 study with updated examples and illustrations and none of the errors that marred the first book. It does not assume great previous knowledge of genetics or statistics; throughout, the book has a pleasing balance between theory and practice. Less pleasing is the imbalance in the bibliographic citations in the various chapters; some controversial statements are made dogmatically and unsupported by literature citations, whereas others are supported unnecessarily by large numbers of references.

The subject matter covers basic concepts of Mendelian and quantitative genetics in the first five chapters, although a consideration of chromosomes themselves (polyploidy, aneuploidy and haploidy) is deferred until the last (nineteenth) chapter. The remaining chapters are the core of practical tree improvement covering the four most common approaches to tree improvement: individual tree selection and breeding; provenance testing; species and racial hybridisation; introduction of exotics. A useful glossary and adequate index are provided.

This book holds something of value to all foresters and crop breeders. It is a basic reference for tree breeders and essential for all courses in forest genetics and tree improvement.

J. Burley

Nuclear magnetic resonance

NMR in Biological Research: Peptides and Proteins. By Kurt Wüthrich. Pp. xii+379. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1976.) \$45.95; Dfl.115.

THE increasing interest in the application of nuclear magnetic resonance (NMR) techniques to biological problems is reflected in the spate of books and review articles which have recently appeared on the subject. Professor Wüthrich's book is a valuable addition to this collection in that he has produced an ideal text for introducing the chemist or biochemist into this area of research. The main emphasis of the book is on illustrating the different kinds of biologically relevant information which can be

obtained from NMR methods. By restricting himself to studies of peptides and proteins he has been able to present a comprehensive and coherent account of the potential of the technique in these systems. Almost all the principles of NMR biological applications are contained in such studies so the student should have little difficulty extending them to other systems.

The book deals with the theoretical aspects of NMR in an elementary phenomenological fashion and provides the key references required to provide more detailed knowledge in these areas. The text covers studies of structure, conformation and molecular dynamics of peptides and proteins, ^{13}C , ^{15}N and other nuclei studies related to these systems, and also considers the effects of paramagnetic centres on proteins. Although no attempt is made to present a comprehensive coverage of the literature, several well-chosen examples are discussed in detail for each of the applications considered. Many of these examples are from recent work in Professor Wüthrich's laboratory. In particular, his excellent ^1H spectra of basic pancreatic trypsin inhibitor have been used to good effect to illustrate a wide range of applications in protein NMR studies.

The book contains a good deal of

useful NMR reference data for amino acids, peptides and proteins. The author has paid special attention to ^{13}C (50p) and ^{15}N (23p) biological applications — areas inadequately covered in other texts. I recommend this book to anyone interested in assessing whether NMR can help with their biological problems.

J. Feeney

Blood vessels in perspective

Blood Vessels. (Biological Structure and Function.) By W. J. Cliff. Pp. ix + 214. (Cambridge University: Cambridge and London, April 1976.) £10.

THE object of this monograph is to show how the different anatomies of the different types of blood vessel are related to their several functions. In addition, an attempt is made to deal with the alterations in wall structure and function that result from pathological processes. It is partially successful.

The author works in the Department of Experimental Pathology at the Australian National University, and his background enables him to look at blood vessels in pretty broad perspective. The result is a useful mix of structure and function, although structure gets the stronger emphasis. Certainly a broad view is necessary when attempting to review in depth the anatomy, ultrastructure, physiology, pharmacology and pathology of all types of blood vessels; the author admits that no one person could carry out the task to perfection. Thus, each specialist will feel some dissatisfaction when he reads the parts which deal with his own field—a wrong emphasis here, an important point missed there, and sometimes a superficial treatment which betrays misunderstanding. These weaknesses are more apparent when the author discusses function.

The main chapters deal with the cellular, extracellular and ancillary structures connected with the walls of blood vessels, but there are also chapters dealing with the vessels as functional units. Extensive use of comparative studies is made to indicate the way vascular structure and function have been developed. The book contains 67 figures, the majority of which are half-tone photographs of histological sections and electron micrographs. There are also 34 pages of references, the most recent being from 1972 journals.

For a refreshing and overall view of blood vessels, the book has much to commend it. The author has a pleasing style of writing, which lends itself to easy reading. The cover states that the book is aimed primarily at anatomists, physiologists and pathologists: they will learn and derive enjoyment from reading it.

I. C. Roddie

Water facets

Water: A Comprehensive Treatise. Edited by Felix Franks. Vol. 4: Aqueous Solutions of Amphiphiles and Macromolecules. Pp. xxi + 839. Vol. 5: Water in Disperse Systems. Pp. xvi + 366. (Plenum: New York and London, 1975.) \$44.50 each volume.

THESE volumes bring to a close the monumental compendium on water which Felix Franks has brought into being with the aid of two dozen or so associates. With this treatise in hand, the reader can certainly get an initial grip on the literature of almost any facet of this multidimensional field, although many of the chapters leave one less than satisfied with the current level of understanding of the problem. This is, however, more a reflection of the overwhelming complexities of the potential interactions of water with the materials of the inorganic, organic and biological worlds, than on the authors of this work.

Volume 4 and 5 differ from their predecessors in point of view; here water is no longer the subject, but rather the all encompassing 'environment'. Students of biological macromolecules have considered it natural to examine them in water as the native habitat, and have only lately come to realise that many of the properties of these macromolecules actually arise in large part as a *consequence* of their existence in the aqueous milieu. On the other hand, the field of synthetic polymer chemistry has developed primarily in organic solvents, and thus in this area the role of water in changing polymer properties and conformations is both explicitly better discriminated, and less well explored.

Volume 4 opens with a chapter by Felix Franks on Hydrophobic Interaction, summarising the theoretical and experimental approaches to small molecules in water which have been taken in an attempt to understand hydrophobic bonding as it might stabilise macromolecular conformations. This serves as a useful complement to the long chapter by Eagland on Nucleic Acids, Peptides, and Proteins, in which is assembled an enormous volume of information on these macromolecules as they exist in aqueous solutions. The merging of these approaches is largely left to the reader in this volume, although it might be worth pointing out that these authors have recently combined forces to bring about an excellent synthesis in an article in *Critical Reviews of Biochemistry* (3, 165, 1975).

Volume 4 also contains thorough summaries of the present status of our understanding of Lipids by Hauser, and of Polysaccharides by Suggett. The

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remainder of this volume comprises chapters on synthetic systems. That on Surfactants by Kresheck provides a nice summary of micelles and micelle-forming molecules; the chapter on Dye-stuffs by Duff and Giles emphasises the spectroscopic effects of dye aggregation and stacking interactions, and as such provides a useful and less frequently presented starting point for considerations of the interactions involved in 'stacking' nucleic acids, etc. The final chapter on Synthetic Polymers, by Molyneaux, helps us to organise this vast literature.

In some senses volume 5, which deals largely with what seem to be totally intractable systems, represents the more unusual of these volumes. It opens with a detailed but well presented chapter on Colloidal Systems, again by Eaglund, which pulls together many aspects of the present state of colloid chemistry. The chapter by Clifford on Water in Capillaries and Thin Films, is, as one might expect, mostly descriptive. It presents, however, one of the most balanced and coherent summaries I have read of two very controversial areas: the status of anomalous water ('polywater'), and of water in cells and tissues. Forslind and Jacobsson use the chapter on Clay-Water Systems in part to provide us a summary of their point-of-view on water structure and water-surface interactions. The remainder of the volume is devoted to chapters on water in very special environments: including Foams and Emulsions (Phillips), Solid Surfaces (Zettlemoyer, Micale and Klier) and Biopolymer Surfaces (Berendsen). Here, as one might expect, the behaviour of water is primarily modulated by the molecular properties of the material comprising the surface or interface at issue, and less by the properties of bulk water.

Overall, Franks and his colleagues have done well by us, providing an appreciation of the ubiquitous effects of water in controlling almost all of life processes, and at the same time providing us with a point at which to approach the various problems of concern to individual investigators. This treatise should be available to all serious research workers concerned with chemical or biological problems in a primarily aqueous world.

Peter H. von Hippel

Crystallinity of macromolecules

Macromolecular Physics. Vol. 2: Crystal Nucleation, Growth, Annealing. By Bernhard Wunderlich. Pp. xi+461. Academic: New York and London, March 1976.) \$46.50; £25.60.

THIS is the second volume in a series so far devoted to phenomena associated with the crystallinity of macromolecules. Volume 1 having dealt with structural aspects, the present volume covers the crystallisation process itself in three consecutive chapters headed by the three subtitles of the book. The book has been long awaited in response to a very real need. This is a work primarily for the active researcher in the field but should also be most useful as a reference book for wider readership in search of information. The book follows no traditional pattern as regards the detailed subject matter or its organisation, and as such is without rival. Its function, as I see it, is intended to be twofold: compilation of knowledge as it exists at present; placing this knowledge in the framework of fundamentals.

As regards my first point, the book is of inestimable value for anybody involved in the subject. Much of the information contained is outside the scope of the usual 'handbook' classification and simply cannot be obtained elsewhere except for the widely scattered original papers. As in volume 1 the Tables are particularly of value in this respect and so is the comprehensive bibliography giving full titles. Also for a book of this size the information content is remarkably up to date.

In addition, the unconventional juxtaposition of an unusually wide range of different subjects in support of particular themes is both informative and stimulating. For example, it contains material on liquid crystals, on macroscopic polymer crystals obtained from polymerisation in the solid state, on simultaneous polymerisation and crystallisation (a particularly unique feature of the book), on organic materials, and even on elements forming polymers—all subjects which although relevant, normally do not feature in the standard polymer textbooks.

To achieve the second purpose the author goes back to the fundamentals of phase transitions before his treatment of polymers. Whether these extensive introductions would stand their ground as independent treatises may well be questioned. Even so, they represent a laudable endeavour for a

book on polymers. It is a further question how long the present theoretical framework will remain accurate, or even pertinent, as the subject is still in a state of development; there have been changes even since the book went to the press. Even so, the author is to be praised for his courage in investing all this work in a subject still in a state of flux.

Although, as in volume 1, there is still too much uncritical juxtaposition of facts, there are now certain firm anchor points: altogether a more deliberate attempt has been made to provide critical guidance when presenting conflicting information than in the first volume.

The book is beautifully and carefully produced. As in volume 1, the author has even gone to the trouble of re-drawing diagrams to suit his points and to achieve the high aesthetic standards he set for himself.

Altogether it is a welcome and laudable effort.

A. Keller

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Erratum. In the review of *Lunar Science: A Post-Apollo View* (S. R. Taylor) by S. K. Runcorn (*Nature*, 260, 813, 1976), paragraph 3, line 20 should read: "... implausible—Velikovsky's predictions that ...".

Insect ecology

Insect Ecology. By Peter W. Price. Pp. xii+514. (Wiley-Interscience: New York and London, October 1975.) £8.10.

THIS book is valuable as the only recent, complete text of insect ecology. Other current works stress either insect population dynamics or applied aspects of the subject. A cursory glance is encouraging; the author has read very widely, selected a balanced bibliography (from both sides of the Atlantic) and covered all the major topics one would hope to see, with the possible exception of insect movements (population structure, migration, and so on).

A closer look reveals a high vertebrate content, and initiates a suspicion that this may be not so much an insect ecology text as an ecological compendium, with examples to illustrate each concept selected from insects where possible. This suspicion materialises as being partly unfounded, in that the extensive coverage of the behaviour of vertebrate predators helps us to understand the nature of selection acting on insects in their capacity as prey. In some other chapters, however, the vertebrate content is unnecessary and could

be misleading, since, in cases in which an ecological generalisation can only be illustrated with vertebrate examples, there may be good reason for this. The generalisation may not extend to insects, especially to those with complex life cycles. Some critical evaluation of the extent to which this is true would have been valuable in an insect ecology text.

Some of the chapters have problems which I hope will be corrected in future editions. Here are three examples. The "Reproductive Strategies" chapter persistently implies that fecundity may evolve (in either direction) to balance imposed changes in mortality, a hypothesis which is wrong, unless accounted for in terms of group selection, which it is not.

The "population dynamics" chapter seems to use the words "limit", "control", "regulate" and "determine" interchangeably and without definition. This is a pity, considering the semantic difficulties which have arisen in the past in this field in relation to the use of these words. It results in a very unclear presentation of the 20-yr-old "density-dependence" debate, since one of the causes of dispute stemmed from the fact that members of opposing camps were using the word "control" in different senses. It also results in repeated use of the phrase "density-independent regulation", which, in current usage, is a contradiction in terms.

The chapter on social systems and behaviour seems unnecessarily abstract, since it does not describe the social organisation of any insect. The theoretical content is not well balanced, and sometimes becomes self-contradictory through accepting incompatible conclusions of different authors. For example, the basic hypothesis of group selection is accepted in the second paragraph on p. 319 and rejected at the bottom of the same page.

In sum, this is a very useful book, an excellent source of references, but one in which the theoretical arguments must occasionally be taken with a pinch of salt.

Michael C. Singer

on man, so a book by him on the subject must be of interest to all those concerned with environmental factors. Although on the dust sheet it is proclaimed that this book covers the whole field, and is a comprehensive review of relevant work, it may be doubted whether Professor LeBlanc would make such a claim. The book is quite short, less than 20,000 words, but is lavishly illustrated with graphs, and so on. (There are so many it is not surprising some of the figure legends have been interchanged.)

In the relatively brief text, Professor LeBlanc sensibly devotes a good deal of space to his own work, and this is certainly the most interesting and useful part of the book. He describes the experiments he and his colleagues have carried out on the effects produced by immersion of the face in cold water and comparing these with the changes observed when the hand is put in cold water at about 4 °C. Lewis showed in 1930 that the blood flow in the fingers shut off virtually completely within seconds after immersion in ice water. The fingers became painful and the pain increased for several minutes; then, after 5–6 min, the pain stopped and it felt as if the water temperature must have been raised. This change was due to a sudden vasodilatation and a large blood flow.

Cold-induced vasodilatation is a remarkable phenomenon, and, as Professor LeBlanc has shown, in people who habitually expose their hands to cold water, the vasodilatation comes on more rapidly and is larger than in unaccustomed subjects. Heart rate and blood pressure increase as pain increases, and return to control values during vasodilatation. In contrast, when the face is immersed in water at 4 °C there is pain and an increase in blood pressure, but heart rate slows. This effect is not observed if the face is immersed in warm water at, say, 30 °C. There is no change in heart rate or blood pressure, and there is no pain. The bradycardia in the cold can be evoked by cold air as well as cold water. Professor LeBlanc suggests that the pain and distress often experienced by patients recovering from an attack of coronary heart disease when they walk out of doors on a cold day facing the wind could be due to this bradycardia combined with an increase in blood pressure.

It is undoubtedly useful to have a detailed account of the work of Professor LeBlanc, and for this reason the book is recommended. It is not a complete review of what is by now a very large body of research work dealing with the effects of cold on man.

O. G. Edholm

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Effects of cold on man

Man in the Cold. (A Monograph in the Bannerstone Division of American Lectures in Environmental Studies.) By Jacques LeBlanc. Pp. x+195. (Thomas: Springfield, Illinois, November 1975.) \$15.50.

PROFESSOR LeBLANC is well-known for his studies on the effects of cold